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ERRATA

The Annals of Applied Biology, 32, 4

(1) Page 289 Table 6 column 2
(2) Page 293 Table 11 column 2

Year *after* fallow should read Year *before* fallow

The nature and distribution of various forms of nitrogen in the potato

BY H. E. STREET, A. E. KENYON AND G. M. WATSON
University College, Nottingham

(With 7 Text-figures)

The distribution of nitrogen fractions in the organs of King Edward potato plants harvested at intervals during the growing season has been determined. A general picture of the nitrogen distribution is presented and certain problems suggested by the data are discussed.

Each organ of the plant shows a uniformity in its nitrogenous composition during the whole season of active growth. The seed tubers are characterized by their high content of non-protein nitrogen. This fraction yields the greater part of the nitrogen withdrawn from the seed tubers; the amide, amino and 'other nitrogen' fractions all being markedly depleted. The severely depleted tubers retain some 20% of their initial nitrogen content.

The roots are rich in non-protein nitrogen, in which nitrate nitrogen is an important fraction. The roots from ammonium sulphate-treated plots had a higher total nitrogen content than those from untreated plots, due to accumulation of ammonia nitrogen.

The 'tops' have a higher nitrogen content and a higher proportion of protein than the roots. There is an increase in protein content and a decrease in non-protein nitrogen in passing from stems, to petioles, to leaf laminae. The depleted tubers and roots and stems have a uniform low-protein content of the order 0·4–0·6 mg. protein nitrogen per g. fresh weight.

Asparagine and glutamine occur in approximately equal amounts in the seed tubers and roots. Glutamine is more completely withdrawn than asparagine from the tubers, and in the 'tops' it constantly exceeds asparagine in amount.

A study of the concentration gradients, from leaves to petioles, to stems, to stem bases and new tubers, of the fractions of the non-protein nitrogen focuses attention upon the 'other nitrogen' as containing the organic nitrogen most actively involved in translocation.

The concentration and total content of the glycoside solanine in the different organs has been determined at each sampling.

INTRODUCTION

A study of the nature and distribution of various forms of nitrogen in the potato plant during growth under field conditions has been undertaken during the period 29 April to 28 August 1944. The object of these experiments was to gain a broad general picture of the nitrogen distribution from setting to tuber formation, which would serve as a background to subsequent studies of the effect of various manurial treatments on the nitrogen metabolism.

EXPERIMENTAL METHODS

General plan

Sprouted King Edward tubers were planted in four plots on 29 April. Each plot contained thirty-five tubers planted in rows 30 in. apart, with 24 in. between each tuber along the rows. Each tuber was weighed and numbered at the time of planting, and specimen tubers selected at random were removed for analysis. During the growing season plants were removed from the plots in the early morning and immediately weighed, dissected into their separate parts (tops, old tubers and roots in the early samplings; leaves, petioles, stems, roots and new tubers in the later samplings) and the different parts weighed and analysed.

Two of the plots received a dressing of ammonium sulphate (10 oz. per sq.yd.). No difference was evident in the appearance or tuber yield of plants from these plots as compared with the two untreated plots.

Method of drying and extracting the plant material

The plant material was immediately cut up and distributed in two weighed portions. The first was dried by heating in an electric oven for 1 hr. at 90° C. and then overnight at a temperature between 50 and 60° C. in a rapid current of air from an electric fan attached to the drying oven. The loss of weight on drying gave the moisture content. The weighed, dry residues were finely powdered and used for the total nitrogen determinations.

The second portion of fresh tissue was immediately frozen at −10° C., stored overnight at this temperature, minced in the frozen condition and weighed amounts taken for the preparation of the aqueous extracts for estimation of the different fractions of the soluble nitrogen.

20 g. of frozen mince were mixed with sufficient

water to give a total volume of 100 ml., and the resulting tissue/water mixture shaken for ½ hr., transferred to a tincture press and the resulting extract strained through calico. Each 5 ml. aliquot of extract was taken as equivalent to 1 g. fresh weight of plant tissue. This method, which has been previously studied in detail by Steward & Street (1945), gave consistent analytical results using tissue/water mixtures up to a strength of 40 g. fresh tissue per 100 ml. mixture, except in the extraction of the roots which are markedly fibrous and not effectively disintegrated by the mincing. The method was therefore modified for root extracts by pulping the minced roots in a mortar and using only 10 g. fresh weight per 100 ml. of mixture.

Total nitrogen. Samples of powdered dried tissue, ranging from 20 to 50 mg. according to nitrogen content, were digested by the Reduced Iron method of Pucher *et al.* (1930), adapted to a micro-Kjeldahl scale. This method ensures nitrate reduction. The digests were distilled in a Parnas-Wagner micro-Kjeldahl distillation apparatus and the ammonia absorbed in 5 ml. *N*/50 sulphuric acid and the excess titrated against *N*/100 sodium hydroxide using Tashiro's indicator. The results are expressed as mg. nitrogen per g. fresh weight.

Total water-soluble nitrogen. The nitrogen content of 1 ml. aliquots of the aqueous extracts was determined as described under total nitrogen.

Total non-protein nitrogen. The aqueous extracts, immediately after removal of the aliquots for the determination of total water-soluble nitrogen, were heated rapidly to 80° C., maintained at that temperature for 10 min., rapidly cooled, the water lost by evaporation replaced, and the mixture filtered. The nitrogen content of 1 ml. aliquots of the filtrate was determined as described under total nitrogen. This method of removing heat-coagulable protein is that recommended by Vickery *et al.* (1935*a*). The filtrate obtained was used for the determination of ammonia nitrogen, the amide-nitrogen fractions, and amino nitrogen.

Heat-coagulable nitrogen. The total water-soluble nitrogen minus the total non-protein nitrogen is designated heat-coagulable nitrogen.

Protein nitrogen. The total nitrogen minus the total non-protein nitrogen is designated protein nitrogen.

Ammonia nitrogen. Ammonia nitrogen was determined by distillation *in vacuo* at 40° C. according to the method of Vickery *et al* (1935*b*), using a quantity of extract equivalent to 2 g. or more of fresh tissue. The ammonia was absorbed in 5 ml. of *N*/50 sulphuric acid, and the excess acid titrated against *N*/100 sodium hydroxide, using Tashiro's indicator.

Total amide nitrogen. Total amide nitrogen was determined by the method of Vickery *et al.* (1935*b*) using a quantity of extract equivalent to 1 g. or more of fresh weight of the tissue. The ammonia liberated by hydrolysis was determined as described under ammonia nitrogen.

Glutamine amide nitrogen. Glutamine amide nitrogen was determined by estimation of the ammonia liberated by the mild hydrolysis adopted by Vickery *et al.* (1935*b*). The ammonia liberated was determined as described under ammonia nitrogen.

Asparagine amide nitrogen. The total amide nitrogen minus the glutamine amide nitrogen is designated asparagine amide nitrogen. The values for glutamine nitrogen and asparagine nitrogen are obtained by doubling the respective amide nitrogen values.

Amino nitrogen. Amino nitrogen was determined in the Van Slyke micro-amino-apparatus (Van Slyke, 1913, 1915) using a reaction time of 10 min. Extract yielding 0·7–2·0 c.c. of nitrogen gas, freed from ammonia, if necessary, and acidified with acetic acid, was used for each test. If undue frothing occurred when the reaction chamber was shaken, 1 drop of toluene was added to the extract before it was drawn into the reaction chamber. The value obtained was corrected for the reactivity of the amide group of glutamine, by subtracting from the Van Slyke value 80% of the glutamine amide-nitrogen value. The amino-nitrogen value so obtained includes the amino nitrogen of both asparagine and glutamine.

Amino-acid nitrogen. The amino nitrogen determined as described above minus the total amide nitrogen is here designated 'amino-acid nitrogen'. This value is a measure of the amino nitrogen of the amino acids, but it should be emphasized that the non-amino nitrogen of the free amino acids will be included in the fraction 'other nitrogen'.

Nitrate nitrogen. The residue obtained from the total amide-nitrogen determination after removal of the ammonia formed by hydrolysis, was transferred to the Parnas-Wagner micro-Kjeldahl distillation apparatus, treated with 0·25 g. Devarda's alloy, a very small piece of paraffin wax and 12 ml. of 40% w/v solution of sodium hydroxide and distilled for 10 min. The ammonia, formed by reduction of the nitrate, was determined as described under total nitrogen.

Other nitrogen. The total non-protein nitrogen minus the sum of ammonia, amide, amino and nitrate nitrogen is here designated 'other nitrogen'.

Total solanine. The total solanine (solanine plus solanidine) was determined by the method of Rooke *et al.* (1943) using the Spekker Absorptiometer with no. 605 yellow-green filter, and diluting solution S, if necessary, so that the final coloured solution did not contain more than 1 mg. total solanine per 10 ml.* The sample of solanine initially used as a standard was kindly supplied by Dr L. H. Lampitt.

* We wish to acknowledge the kind help of Dr W. F. Elvidge of Boots Pure Drug Co. Ltd., who has carried out all the colorimetric determinations on solutions S submitted by our laboratory.

EXPERIMENTAL RESULTS

The preliminary experimental results here reported are shown in Figs. 1–7.

Fig. 1 shows the average weights of whole plants, excluding the depleted tubers, and of 'tops', roots and new tubers at each sampling time during the growing season. The extreme limits of weight within each sample are shown for all the samples containing five or more plants.

Figs. 2–4 give the distribution of the various nitrogen fractions in the different organs of the plant. The average and extreme values for each fraction observed throughout the season are indicated by the convention adopted in Fig. 1. From each complete analysis made the total content of each fraction in the different plant organs can be calculated. A general picture of the total content of each nitrogen fraction in 'tops' and roots can be obtained by a combination of the data presented in Figs. 1–4.

Fig. 5 illustrates the concentration gradients of selected nitrogen fractions in one plant from the leaves downwards to the stem bases and runners and, at a later sampling date, in a second plant from the leaves to the new tubers.

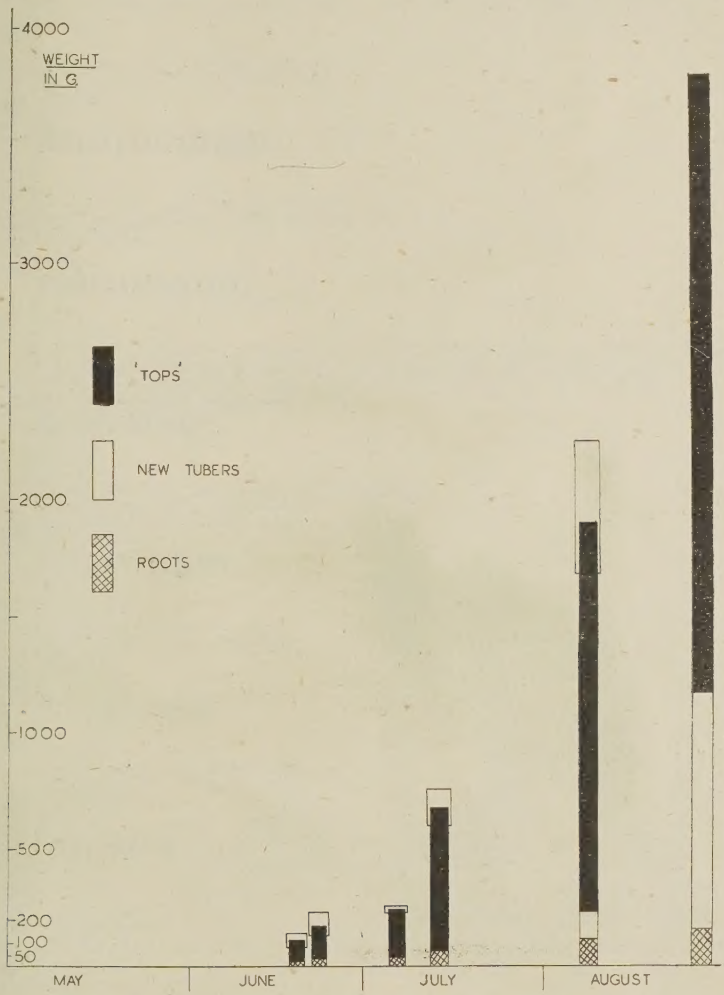

Fig. 1. Weight of King Edward plants, excluding the depleted tuber, taken for analysis at intervals from 19 June to 28 August 1944 (tubers set on 29 April). Average weight at each sampling shown. The limits of variation in individual plant weights shown by means of the superimposed rectangles, when not less than five plants were weighed in each sample.

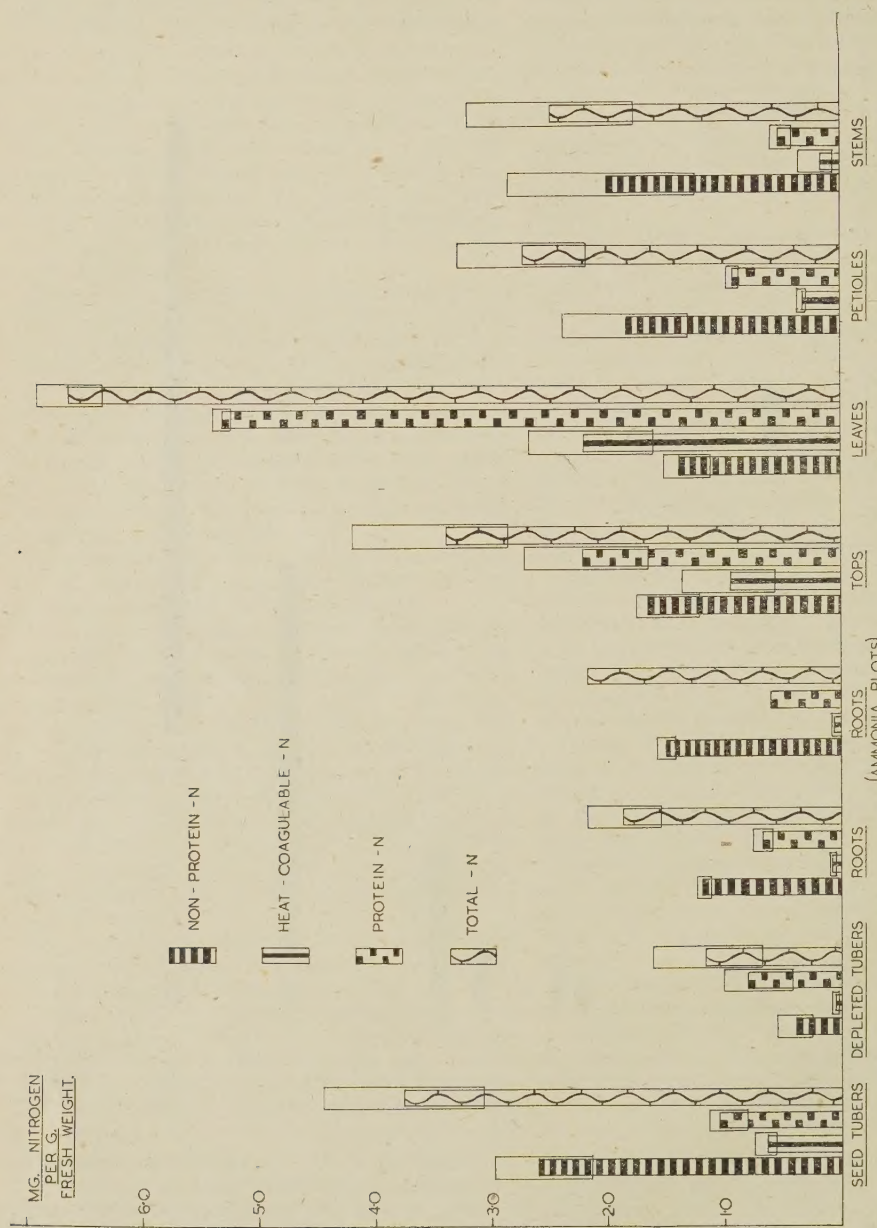

Fig. 2. Content (as mg. nitrogen per g. fresh weight) of total nitrogen, total protein nitrogen, heat-coagulable nitrogen and non-protein nitrogen in the seed tubers, depleted tubers, roots (those from plants grown on untreated plots and on plots receiving dressings of ammonium sulphate shown separately), 'tops', leaves, petioles and stems. The average value for each fraction and the limits of variation observed throughout the growing season shown as in Fig. 1. 'Tops' analysed throughout the season; leaves, petioles and stem analysed separately during the period 23 June to 28 August.

Fig. 6 shows the concentration of solanine plus solanidine in each organ, and Fig. 7 the total content in each organ of plants collected at intervals during the growing season. Since the greater part of this fraction is solanine ($C_{45}H_{73}O_{15}N$) the results shown can be converted to mg. solanine nitrogen by multiplying by the factor 0·016.

Some difficulty was experienced in completely recovering the finer parts of the root system of these plants grown in the field. For this reason and

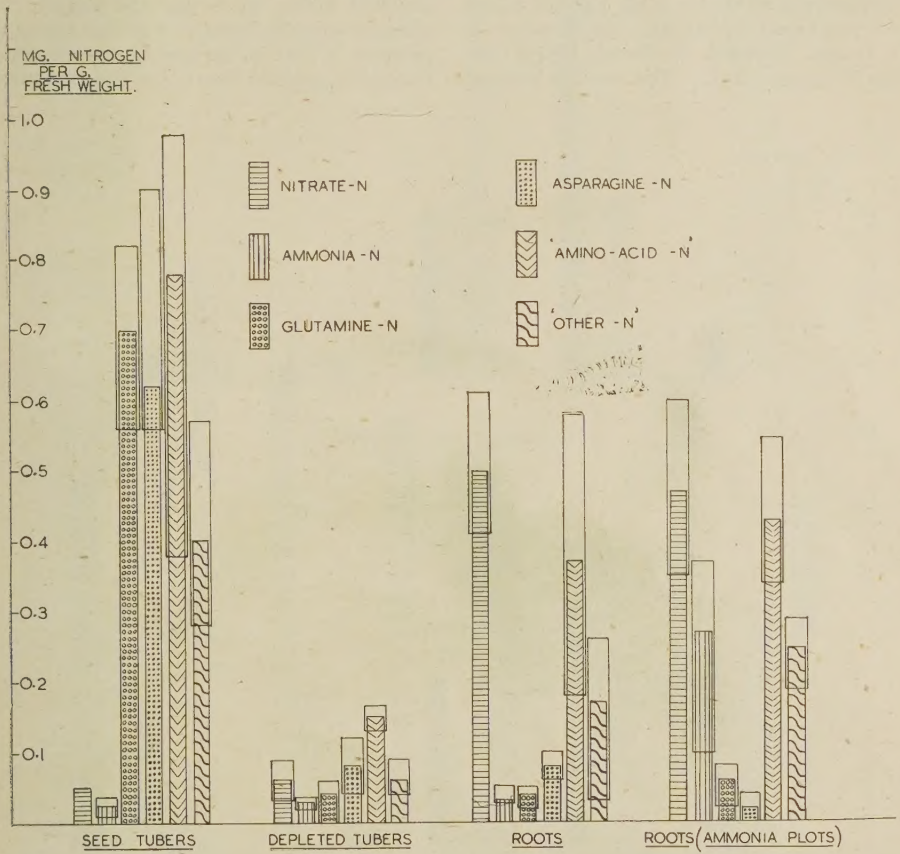

Fig. 3. Content (as mg. nitrogen per g. fresh weight) of the different fractions of the non-protein nitrogen in the seed tubers, depleted tubers and roots (those from plants grown on untreated plots and on plots receiving dressings of ammonium sulphate shown separately). The average value for each fraction and the limits of variation observed throughout the growing season as in Fig. 1.

Discussion

The general course of growth during the experimental period of 120 days is shown in Fig. 1. The variation in plant weight (excluding the weight of the depleted tuber) between the individual plants of any one harvesting is more marked during the early stages of growth owing to the influence of such factors as tuber sprouting, weight of tuber and depth of planting. Less variation occurs between individual plants later in the season. No noticeable difference in size and general appearance occurred between plants from the plots treated with ammonium sulphate and those from the untreated plots. The general form of the growth curve is similar to that recorded by various workers for other plants.

because whole tubers were set (and therefore their content of the various nitrogen fractions can only be calculated from the average values for the samples of seed tubers analysed), the course of nitrogen absorption and of new protein synthesized during the growing period is not here presented. A general picture of nitrogen absorption, of increase in total content of each nitrogen fraction and of distribution in the 'tops', roots and tubers can, however, be built up from the data presented in Figs. 1–4. A detailed picture of the course of nitrogen absorption should

be available from sand cultures now under investigation.

Each organ shows a basic uniformity in its nitrogenous composition during the whole season of active growth. A decrease in protein content of the leaves only becomes evident when tuber formation is at its height.

The composition of resting King Edward tubers has been previously investigated by Steward & Preston (1940), Steward & Street (1945) and Neuberger & Sanger (1942). The present analyses whilst the nitrogen supplied from the tuber per g. of plant produced falls from 1·44 to 0·96 mg. These results confirm that the tuber retains some 20% of its initial nitrogen content even at an advanced stage of growth, and also illustrate the decreasing role of the seed tuber as a source of nitrogen as growth progresses. The complete nitrogen analyses of the depleted tubers show that the simpler forms of nitrogen are withdrawn to a greater extent than the proteins. Whilst in the seed tubers the non-protein fractions constitute 68–71% of the total nitrogen, in

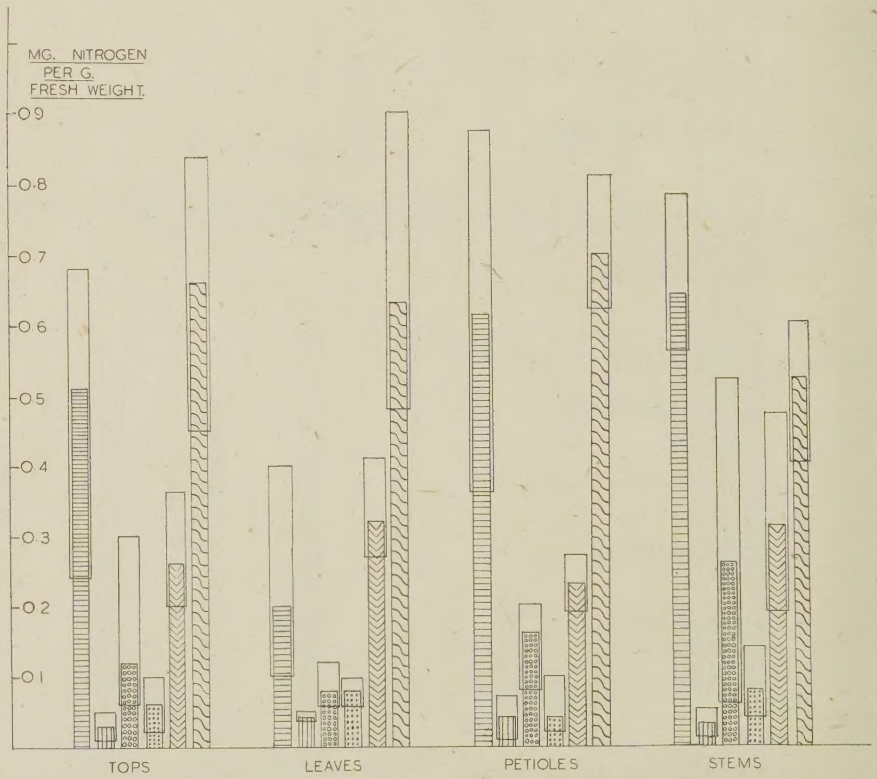

Fig. 4. Content (as mg. nitrogen per g. fresh weight) of the different fractions of the non-protein nitrogen in the 'tops', leaves, petioles and stems. Key as in Fig. 3.

of the seed tubers confirm this earlier work. Denny (1929, 1930) has recorded the decrease in total nitrogen content of the seed tubers of Bliss Triumph and Irish Cobbler from the state of emergence of the sprouts to flowering. The depleted tubers of Bliss Triumph retained 41% of the original nitrogen content when the plants were 10 in. high and 24% at flowering. The corresponding values for Irish Cobbler were 25 and 17%. In the present investigation with plant weights ranging up from 119 to 285 g. the percentage of its original total nitrogen content retained by the tuber falls from 43 to 18%, the depleted tubers they account for only 27·5–39% of the total nitrogen. The greater part of the loss of total nitrogen is due to withdrawal of the non-protein fractions, as is shown by the fact that the average loss of total nitrogen is 2·6 mg. per g. fresh weight, and of this 2·2 mg. is contributed by the non-protein fractions. There is no significant decrease in the nitrate and ammonia concentrations initially present, so that in the depleted tubers they constitute a higher percentage of the non-protein nitrogen. In the seed tubers the ammonia nitrogen and the nitrate nitrogen constitute respectively 0·9

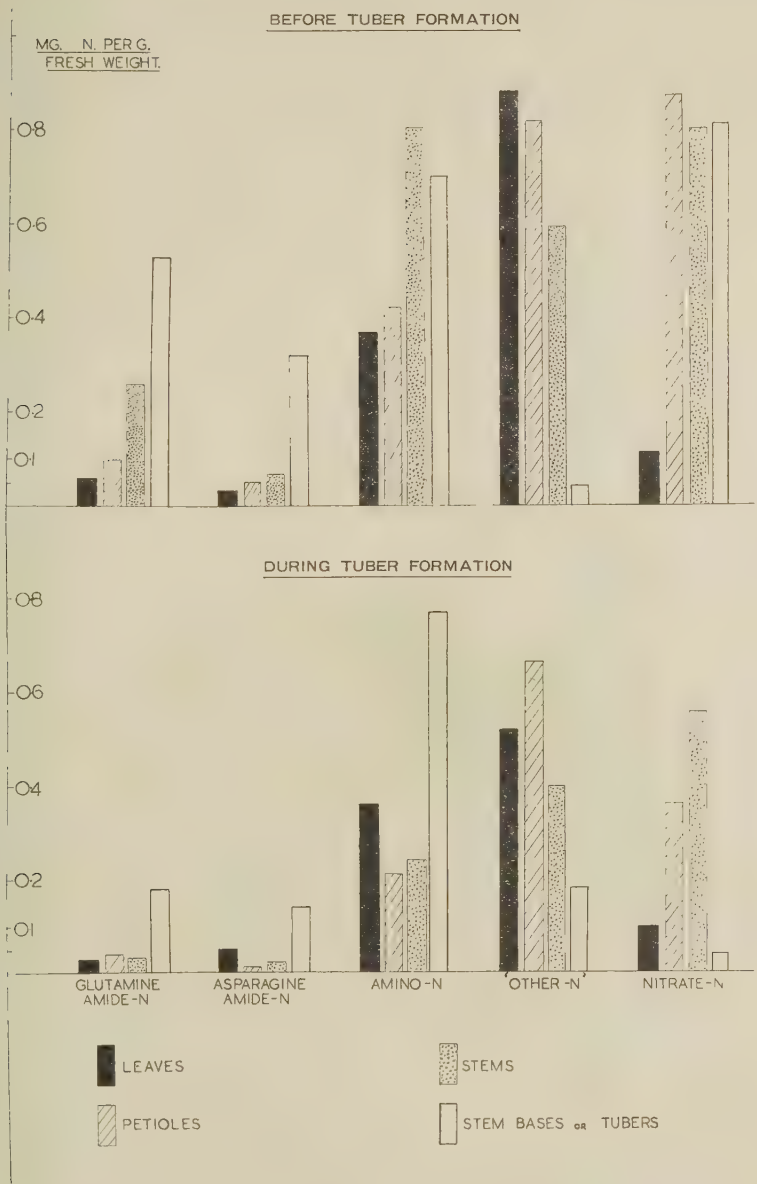

Fig. 5. Gradients of concentration of various nitrogen fractions existing in plants collected on 6 July and 28 August. The plant collected on 6 July yielded 15 g. blanched stem bases and runners; the plant collected on 28 August yielded 1010 g. new tubers.

and 2·0% of the non-protein nitrogen; the corresponding values in the depleted tubers are 7·0 and 14·5%. The amino-acid nitrogen constitutes 30%, the 'other nitrogen' 15% and the total nitrogen of the amides 52% of the non-protein nitrogen of the seed tubers, the corresponding values for the depleted tubers are 35, 14·5 and 29%. These three fractions of the non-protein nitrogen are therefore heat-coagulable nitrogen during withdrawal of nitrogen from the seed tuber may exceed the total loss of protein nitrogen, suggesting that whilst some of the heat-coagulable nitrogen has been broken down to simpler forms and transported to the growing parts, a fraction of it has been simultaneously converted to protein not extractable from the tissues by water.

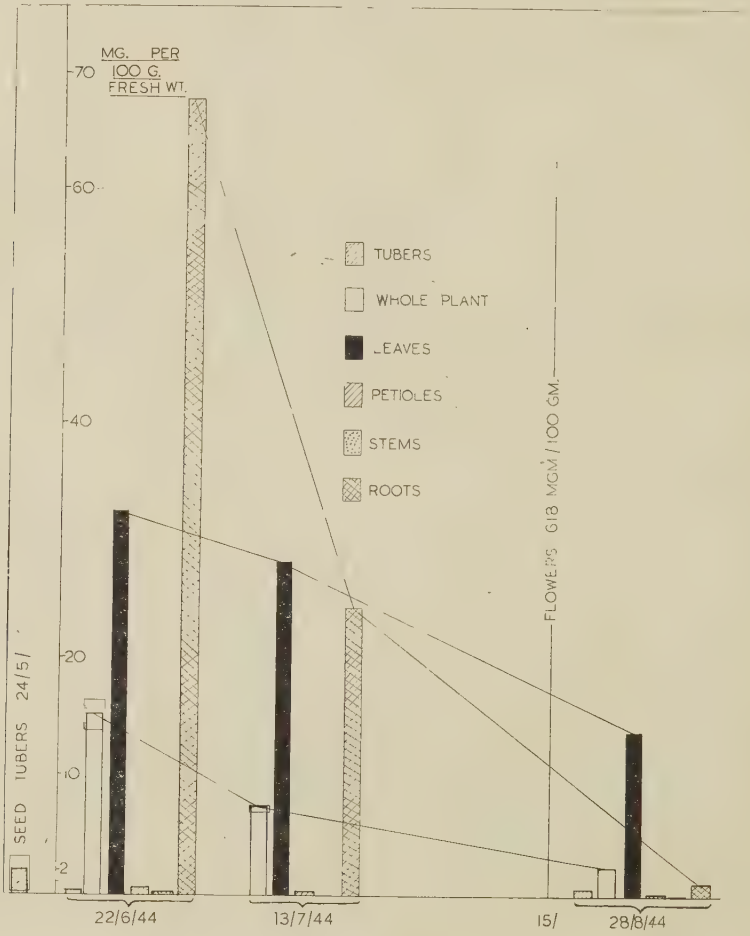

Fig. 6. Concentration of total solanine (solanine plus solanidine) in the various parts of the plant during the growing season.

all mobilized, the drain on the amide fraction being most marked. Both amides are involved, but the withdrawal of glutamine is more complete, so that in some analyses no glutamine could be detected in the depleted tuber, although it was present to the same nitrogen concentration as asparagine in the seed tubers. The content of heat-coagulable nitrogen in the depleted tubers is always very low. The fall in

The roots contain from 1·54 to 2·16 mg. nitrogen per g. fresh weight and of this the non-protein nitrogen constitutes some 63–69%. The roots from plants grown in the ammonium sulphate plots were distinguishable from those from the untreated plots by their higher content of ammonia nitrogen (0·10–0·37 as compared with 0·03–0·05 mg. ammonia nitrogen per g. fresh weight). The ammonia-treated

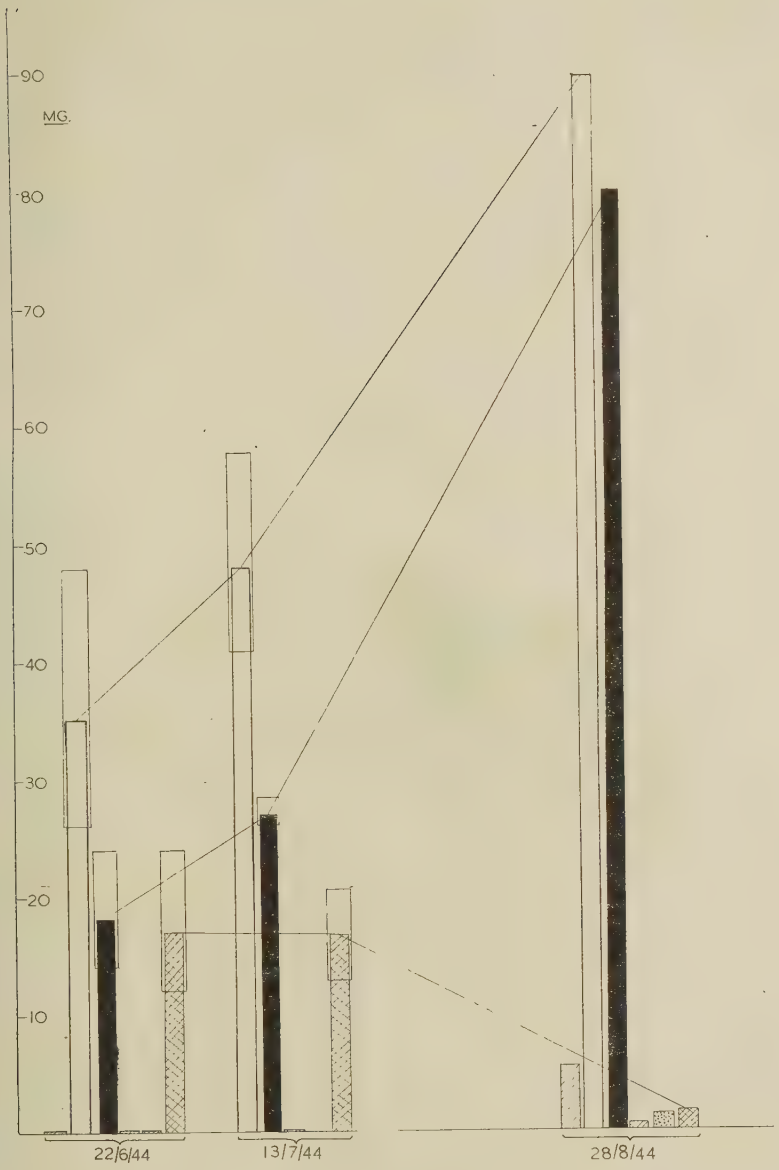

Fig. 7. Content of total solanine (solanine plus solanidine) in each plant organ during the growing season. Key as in Fig. 5.

roots, however, were no less rich in nitrate nitrogen than the untreated roots, the values recorded ranging from 0·35 to 0·60 and from 0·41 to 0·61 mg. nitrate nitrogen per g. fresh weight respectively. The other fractions of the non-protein nitrogen were similarly unaffected, and consequently the ammonia-treated roots were marked by a higher nitrogen content—the average value being 2·14 mg. per g. fresh weight as compared with 1·87 mg. for the untreated roots. The accumulation of ammonia was not accompanied by accumulation of amides, and in both types of root the total amide nitrogen only constituted 2·7–5·1 % of the non-protein nitrogen. These results on the effect of ammonia feeding require confirmation from experiments under carefully controlled conditions before their theoretical implications can be discussed. This problem is at present being investigated by water cultures.

In the earlier samplings the 'tops' were analysed, but later, when the plant size made this possible, the leaves, petioles and stems were analysed separately, and from these results the 'tops' analysis was calculated. The 'tops' are richer in nitrogen than the roots, the total nitrogen content ranging from 4·19 to 3·4 mg. per g. fresh weight up to tuber formation and then falling to 2·84 mg. per g. (28 August 1944). The higher nitrogen content is due to the greater content of protein nitrogen; the average for 'tops' being 2·2 mg. per g. as compared with 0·65 mg. per g. for the roots. The non-protein nitrogen of the 'tops' therefore constitutes only 41·5–48 % of the total nitrogen. The content of nitrate nitrogen is comparable with that of the roots: 0·24–0·68 mg. per g. The ammonia content fell between the limits 0·01–0·05 mg. per g. in all the plants analysed. The amide fraction is of interest in that, whilst it does not significantly exceed the concentration found in the root, it shows in contrast to seed tuber and root a predominance of glutamine over asparagine, the glutamine : asparagine ratio ranging from 1·5 : 1 to 4 : 1. We have drawn attention to the withdrawal of 'other nitrogen' from the seed tubers. The concentration of this fraction is always markedly higher in the 'tops' than in the roots, ranging from 0·45 to 0·87 mg. per g. as compared with 0·19–0·29 mg. per g. in the roots.

Comparison of the analyses of leaves, petioles and stems emphasises the very high total nitrogen and protein contents of the leaves and that these fractions decrease through the petioles to the stems. The protein content of the stems is comparable with that of the roots. This lower limit of protein content which is reached in stems, roots and depleted tubers is of the order 0·4–0·6 mg. protein nitrogen per g. fresh weight.

The leaves are characterized by their high protein and low non-protein nitrogen; the stems by their low protein and high non-protein nitrogen. There is an increase in protein content and a decrease in non-protein nitrogen content in passing from stems, to petioles, to leaf laminae.

The existence of gradients of concentration of the various fractions of the non-protein nitrogen in the tops is shown in Fig. 5 which is based on the data of two analyses made before and after the initiation of active tuber formation. In the first the blanched stem bases and runners (6 July 1944) and in the second the immature tubers (28 August 1944) were analysed as representing the foci to which simple forms of nitrogen are presumably being actively translocated. The concentration gradients recorded are those existing in the plants collected in the morning and the study of diurnal variation must be the subject of subsequent investigation. In both analyses there is an accumulation of amides in the stem bases and runners and in the new tubers. This accumulation is particularly marked in the analysis of 6 July 1944 and this is probably due to the fact that this is an 'ammonia' plant the roots of which contained 0·33 mg. ammonia nitrogen per g. Amide synthesis from ammonia and nitrogen-free precursors probably occurs in the stem bases. Both amides, and particularly glutamine, show a negative concentration gradient; the amide concentration increasing from leaves, to petioles, to stem, to stem bases and runners. The analysis of 28 August 1944 shows accumulation of both amides in the new tubers but no well-marked concentration gradient from leaves, to petioles, to stems is evident. Amino nitrogen similarly shows either a negative or an ill-defined gradient. The gradients of amino-acid nitrogen are similarly inconclusive, showing that the presence of amino nitrogen of amides is not masking any well-defined positive gradient of amino acids. The negative gradient of nitrate nitrogen may indicate that it is being translocated upwards from the roots to the leaves and is suffering reduction and utilization in protein synthesis in the upper part of the shoot system. The existence of negative gradients of amide and amino nitrogen in the cotton plant led Maskell & Mason (1929, 1930), Mason & Maskell (1934) and Mason & Phillis (1934) to regard these as reserve forms of nitrogen rather than as mobile nitrogen. The gradients here described and the marked accumulation of both these fractions in the stem bases and new tubers would support this view. Special interest therefore attaches to the 'other nitrogen' data which corresponds to the 'residual nitrogen' of Maskell, Mason and Phillis. The analysis of 6 July 1944 reveals a well-marked positive gradient of the 'other nitrogen', and the analysis of 28 August 1944 is confirmatory except that the value for the leaf falls slightly below that of the petiole. The evidence therefore agrees with that of these workers and focuses attention upon the 'other nitrogen' as possibly containing the mobile nitrogen compounds involved in translocation. The 'other nitrogen', quantitatively an important fraction of the

non-protein nitrogen in all parts of the plant, is complex in composition and will include polypeptide and peptide nitrogen, purine nitrogen (xanthine, hypoxanthine, guanine and adenine have been detected), nitrogen of simple natural bases (choline, trigonelline, cadaverine and, in immature potatoes, narcotine and acetylcholine have been detected), total solanine nitrogen, and the non-amino nitrogen of arginine, histidine, proline and tryptophane. The solanine nitrogen is quantitatively an unimportant fraction of this nitrogen. The quantitative analysis of the 'other nitrogen' of the potato tuber is at present in progress. The development of methods of analysis, applicable in nitrogen studies, for these groups of constituents is a necessary preliminary to a study of their metabolism. Special interest attaches to the simple natural bases, of which choline is quantitatively the most important in the potato, in view of their universal occurrence in plants, their low molecular weight and their possible significance in alkaloidal synthesis and in biological methylation in general.

Bomer & Mattis (1924) recorded the presence of 2–10 mg. of solanine per g. in normal potato tubers, and noted that the solanine content increased when the tubers were exposed to daylight. The method of estimation used by these workers gives, in our experience, a sticky precipitate of solanine difficult to purify and, as described by Rooke *et al.* (1943), gives variable results. Conner (1937) has also studied the effect of radiations on the solanine content of potato tubers, using a modification of the Bomer & Mattis (1924) method.

Von Morgenstern (1907) has examined the distribution of solanine in the whole plant at flowering, but here again the method of extraction and estimation was unsatisfactory. An interesting qualitative micro-chemical study has been made by Molle (1906). The method of estimation of Rooke *et al.* (1943), which involves the use of Marquis reagent (sulphuric acid—formaldehyde) and is based on the work of Pfankuch (1937), is free from many of the criticisms levelled against earlier methods and, except when applied to the fruits, has given precipitates which have not proved intractable to filtration or purification.

Von Morgenstern (1907) showed that at flowering the concentration of solanine was low in tuber, stem and petiole, but much higher in the roots, runners and leaves, and highest in the flowers. When the haulms began to die down the solanine content of the runners, roots, stems and leaves decreased, but that of the flowers altered only slightly. Lampitt *et al.* (1943) found King Edward tubers to contain 7·5 mg. 'total solanine' per 100 g. fresh weight, which was increased by 8–10 weeks' storage in ultra-violet light to 38 mg. per 100 g. They also confirmed the work of Schowalter & Hartmann (1924) by showing that the 'total solanine' content of the tubers increases during sprouting in the dark, thus demonstrating that light is not directly essential to solanine synthesis. Lampitt *et al.* (1943) have also studied the distribution of solanine in Arran Signet potato plants raised from tubers planted in July and harvested 8–11 weeks after planting. The results obtained with the 11-weeks old plants as compared with the 8-weeks old plants showed an increase in concentration in the flowers (416 mg. % at 11 weeks as compared with 280 mg. % at 8 weeks), leaves (61 mg. % as compared with 55 mg. %) and new tubers (9 mg. % as compared with 2·5 mg. %), and a decrease in concentration in the roots (18 mg. % as compared with 40 mg. %) and stem (2·3 mg. % as compared with 3·3 mg. %). Simultaneous analysis of 'total solanine' and of solanine (by titration of the reducing sugars liberated by hydrolysis) showed that whilst free solanidine may occur in the sprouts of some varieties (no solanidine was present in the sprouts of King Edward), in general in all other parts of the plant only the glycoside solanine is present. To obtain a general picture of solanine distribution it is, therefore, only necessary to employ the 'total solanine' determination.

The results shown in Figs. 6 and 7 relate to the solanine distribution in King Edward potato plants during the growing season and serve to confirm and extend the work of Lampitt *et al.* (1943) on this variety. The seed tubers contained 0·2–3·0 mg. of solanine per 100 g. fresh weight. Depleted tubers recovered on 22 June contained 0·2 mg. per 100 g. The concentration in the roots was initially very high (68 mg. per 100 g.), but fell during the growing season to a low value (1·0 mg. per 100 g. on 28 August). The decrease in solanine concentration was so marked that the total content in the root system fell despite increasing weight. At the first harvesting (8 weeks after planting) 44 to 50% of the solanine was concentrated in the roots, at the last harvesting (17½ weeks after planting) the roots only contained 1·6% of the solanine present. The concentration of solanine in the leaves never reaches so high a level as in the root, but the concentration falls much less steeply during the season (33 mg. per 100 g. down to 14 mg. per 100 g.). Despite the fall in concentration therefore the leaves increase their total content and accumulate an increasing percentage of the total as the season progresses (52% rising up to 89%). The concentration of solanine present in the petioles and stems remains at a very low level throughout the whole season. The concentration in the petioles ranges from 0·6 to 0·2 mg. per 100 g., decreasing as the season advances. The concentration in the stem falls from 0·2 to 0·1 mg. per 100 g. The flowers are exceedingly rich in solanine, containing 618 mg. per 100 g. The total solanine content of the plants increased from 26 mg. (8 weeks) to 89 mg. (17½ weeks), but the average concentration over the whole plant fell from 15·2 to 2·37 mg. per 100 g. The values

recorded for solanine content, if expressed in terms of nitrogen, represent only a very minute fraction of the 'other nitrogen'. Experiments are now in progress to study the effect of various sources of nitrogen, of carbohydrate and of sterols on synthesis of the glycoside.

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Some causes of chlorosis and necrosis of sugar-beet foliage

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(With Plates 1 and 2 and 1 Text-figure)

The symptoms and characteristics of two virus and one fungus disease and four nutritional disorders of sugar beet which cause chlorosis and necrosis of the foliage are described. The causes of the diseases and methods of distinguishing between them have been investigated by analytical, pathological and field experimental methods.

Experiments in which diagnosis was confirmed by serological and spectrochemical methods show that the two often easily confused diseases, sugar-beet yellows virus and magnesium deficiency, can be visually distinguished.

Sugar-beet yellows virus reduces the potassium, but slightly increases the magnesium content of the leaves.

Magnesium deficiency symptoms are associated with a low magnesium content of the foliage, but may be induced by salt applications without greatly affecting the magnesium analysis.

'Potash' deficiency symptoms are often, but not necessarily, associated with a low potassium analysis and may actually be caused solely by a deficiency of sodium. In the field, symptoms are induced by sulphate of ammonia and phosphate applications and may be prevented in some cases by the application of either salt or muriate of potash, in others by salt only. Some interchangeability of the functions of potassium and sodium in the plant is suggested.

Manganese deficiency symptoms are associated with a low manganese content of the leaves, which can be readily increased by spraying or injection with manganese sulphate solution, but a high concentration of manganese in the foliage, such as sometimes occurs naturally on acid soils, has a toxic effect.

Introduction

Chlorosis and necrosis are symptoms of most disorders of sugar beet. They may result from physical causes, such as drought or water logging; from fungus diseases such as rust (*Uromyces Betae*) and leaf spot (*Cercospora beticola*); from various nutrient deficiencies, such as nitrogen, potash, magnesium and manganese; and from virus diseases, such as those caused by beet yellows and beet mosaic viruses.

Although the characteristic symptoms of many of these diseases have previously been described, e.g. manganese deficiency (Davis, 1939), beet yellows virus (Watson, 1942), beet mosaic virus (Smith, 1934), it was found that for purposes of diagnosis in the field some of these descriptions were confusing or even misleading, for the most obvious and characteristic symptoms of a disease are not necessarily those by which it is most readily distinguished from other diseases. For instance, the leaves of plants infected with beet yellows virus turn yellow, especially between the veins. They become brittle and 'crackly' and develop an apical and intervenal necrosis. This description is adequate to distinguish plants with yellows virus from those with other virus diseases and healthy plants, but will not distinguish them from plants suffering from magnesium deficiency or the later stages of attack by downy mildew. Visual discrimination between these can be made only by attention to details of shade, pattern and texture, and the circumstances in which they occur, which might seem to be insignificant when considering each disease separately. Furthermore, the details of the symptoms produced by the diseases vary considerably with the state of growth of the crop, weather and cultural conditions, and there is considerable difficulty in distinguishing between the diseases, especially on individual plants, when the ranges of symptoms tend to overlap.

This paper describes the symptoms of common diseases causing chlorosis and necrosis of sugar beet and the chemical, plant pathological and field methods which have been used to distinguish between them. These methods are now being used in practical diagnosis of atypical conditions and those complicated by the presence of two or more diseases together. The diseases are those caused by beet yellows and beet mosaic viruses, a secondary complication of downy mildew, magnesium, manganese and potash deficiency diseases and the toxic effect of excess of manganese.

Material and methods

Observations in the field were made in sugar-beet crops in eastern England. Material was usually collected for laboratory treatment from the fields in which observations were made; it was tested for virus by sap inoculation, aphis transmission, or serological methods, and spectrographically for mineral deficiency. Beet mosaic virus is transmissible by sap

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inoculation and by aphides. In sap inoculation the juice from infected leaves is rubbed on to the leaves of healthy plants. In aphis transmission the aphides are fed on the infected leaves and then transferred to healthy plants.

Beet yellows virus is not sap transmissible, but can be transmitted by aphides. Aphis transmission tests, however, are often inconvenient for field diagnosis. They require a regular supply of young healthy plants and aphides, and also, in the autumn and early winter, when most field tests are required, the symptoms may take 3 or 4 weeks to develop, and may be atypical owing to short days and low light intensities.

A more rapid test for the presence of the virus can be made by the use of serological methods. A specific antiserum can be produced by injection of rabbits with sap from infected leaves (Kleczkowski & Watson, 1944). When mixed with sap from other infected leaves in the correct proportions a precipitate is formed, which is not produced when the antiserum is mixed with healthy sap or with sap from plants yellowed from other causes than virus. A positive precipitin test is definite indication of the presence of the virus, but a negative test is not entirely reliable, as it has been found that some infected plants contain materials which inhibit precipitation (Kleczkowski & Watson, 1944).

The deficiency diseases may be identified by the response of the plants to application of the nutrient presumed to be deficient, by chemical analysis, or preferably by a combination of both methods. The nutrients are either applied to the soil, sprayed on to the growing plants, or injected into the plants by the technique elaborated by Roach (1938). In the injection technique, a leaf is removed leaving the petiole attached to the plant, and the cut end of the petiole is inserted into a solution containing the nutrients. Parts of the laminae of the leaves above and below the injected petiole show an improvement in colour or growth when the deficient nutrient is supplied, while the disease symptoms persist on the remainder of the plant.

The chemical analyses were made by means of the Lundegardh apparatus (Lundegardh, 1934; Mitchell, 1936), used with a quartz spectrograph (f_D 60 cm.). The sample to be tested is oven-dried and ground, and 1·25 g. extracted with 50 ml. *N* hydrochloric acid for 24 hr.; this solution is subsequently diluted for the determination of potassium, sodium and calcium. The solution is introduced into the air-acetylene flame of the Lundegardh burner as a fine spray carried by the air supply. The spectrum of the flame is photographed and the concentration of each element estimated by measuring the density of its line on the negative. The content of the different elements is expressed throughout on a dry matter basis.

The analytical method of diagnosis is best applied by combining two procedures: (*a*) comparison of the analysis of diseased and healthy plants from the same locality; (*b*) comparison of the analysis of leaves of the diseased plants with approximate limiting values for the contents of the various elements, derived from analyses of a large number of both healthy and diseased plants. Sometimes diagnosis has to depend on method (*b*) alone, for it frequently happens that both apparently healthy and affected plants from the same field prove to be equally low in the element concerned. But the use of method (*b*) alone has the disadvantage that considerable experience in analytical values is required. Also, the appearance of deficiency symptoms may depend on the contents of other elements besides the one chiefly concerned.

Infectious diseases

Beet mosaic virus

Beet mosaic is a common disease of sugar beet and mangolds (Smith, 1934). It is most frequently found in areas where seed crops are grown. According to Gaskill (1943) in U.S.A., and Gram (1942) in Denmark, it can cause a 35 % loss of seed, and has a serious effect upon the yield of fodder beets, but apparently it does little damage to the sugar-beet root crop. Its distribution and frequency of occurrence correspond with those of beet yellows virus, as it is transmitted by the same vectors, *Myzus persicae* Sulz. and *Aphis fabae* Scop.

The symptoms start with 'vein-clearing', or yellowing of the veins of the young developing leaves. Later the whole plant shows a greenish mottle. The pattern may take the form of 'vein bands', dark green bands following the course of the veins (Pl. 1, fig. 1), or of small light green rings on a darker background (Pl. 1, fig. 2). The patterns are more easily seen by transmitted light. There is no obvious stunting of infected plants, and the leaves do not show marginal necrosis.

Although very common, this disease seems to be relatively unimportant in sugar beet in Britain, but the symptoms should be recognized as those of a distinct disease and not associated with any nutrient deficiency or with beet yellows virus.

Beet yellows virus

Beet yellows is an aphis-transmitted virus disease of great importance in the British sugar beet crop. It varies in intensity from year to year, and those years in which it is most severe always show a serious depression in the mean yield of sugar beet.

The symptoms vary according to whether the infection occurs early or late in the season. If it is early, i.e. between June and August, the first symptoms are usually 'vein-clearing' of the youngest leaves, accompanied by a superficial necrosis which gives the leaves an 'etched' appearance. This may persist on the older leaves, but is usually ephemeral.

As these leaves develop they become yellowed, so that the typical 'yellows' symptoms occur first on leaves of medium age. The chlorosis is very bright yellow, or orange-yellow, and it develops from the tips of the leaves downwards. It is continuous and intervenal, so that the leaves are usually greener at their bases, and around the veins. The yellowed areas pass gradually into the green so that there is no well defined line of demarcation. The chlorotic leaves frequently develop apical and marginal necrosis, with branching lobes projecting between the veins (Pl. 1, fig. 3). The necrotic tissue is golden brown, and may roll at the edges, but does not usually disintegrate. There may be secondary infection with *Sporodesmium* spp., or other fungi. Sometimes the necrosis is in the form of very small spots like freckles, scattered over the chlorotic leaf. In some varieties, e.g. Kleinwanzleben 'E', the 'freckles' may be bright scarlet, and not necrotic. This scarlet colour seems to be diagnostic of virus yellows, for it has not been observed in other chlorotic diseases (Pl. 1, fig. 4).

The degree of stunting of the plants, and the resulting losses in yield depend upon the earliness of the infection. With early infection they are very severe, but plants infected later than the end of August do not usually show any stunting by harvest time. The symptoms start with a chlorotic lesion at the site of the aphis inoculation and often develop asymmetrically (Pl. 1, fig. 5*a*). As the infection becomes systemic, chlorosis develops at the tips of the medium aged leaves (Pl. 1, fig. 5*b*). It is very brightly coloured, sometimes scarlet, and not often combined with necrosis.

Leaves which become chlorotic through virus yellows are thickened and very brittle. When crushed they break into fragments. The surface is at first waxy and glossy, but later becomes dull and dry. The texture and consistency of the leaves is not very useful in diagnosis as many other chlorotic diseases cause thickening and brittleness of the leaves, perhaps not so severe as that of virus yellows, but difficult to distinguish from it in isolated specimens.

The distribution and frequency of the disease depend upon the behaviour of the aphis vectors, and the presence of over-wintering sources of the virus (Hull & Watson, 1945). It is particularly frequent and severe in seed crop areas. It may be evenly distributed throughout a crop, or it may obviously be spreading from some source of aphides or infection; e.g. in circular patches from isolated infected plants in an otherwise healthy crop, or from the side or corner of a field where aphides are entering from an infected source.

As mineral deficiencies are discussed in later sections it is useful here to note the effect of virus infection on the chemical composition of the foliage. In an experiment done in 1942 to determine the effect of different rates and dates of virus infection on the yields of beet sown on different dates, the magnesium content was found to be consistently higher in the infected than in the healthy plants, and the differences varied with the degree and date of infection. The mean for infected plants (averaging all dates and rates of infection) was $0{\cdot}46 \pm 0{\cdot}026$ % as compared with $0{\cdot}53 \pm 0{\cdot}015$ % for healthy plants.

The mean difference in magnesium content between healthy and infected plants is significant, but the greatest differences were found when all the plants were infected on the earliest date, 25 June. In these conditions the early sown infected beet has a magnesium content of 0·62 % compared with 0·48 % for the healthy plants, and the late sown infected beet had a magnesium content of 0·72 % compared with 0·48 %; potassium was reduced in the infected plants, but not significantly; manganese was increased by infection and was also increased by later sowing date.

Similar effects of virus yellows on the magnesium and potassium contents of the leaves can be seen in Tables 2 and 3 (see p. 17), but that on manganese was not confirmed.

Downy mildew (Peronospora Schachtii)

This disease mainly attacks the seed crops and the young root crops. It is spread by air-borne spores and the sources of over-wintering infection are the seed crops, so that it is often associated with virus yellows in the field.

The symptoms start with a loss of colour from the leaves which are attacked. They become thickened and puckered and have a purplish spore-bloom on their under-surfaces. Young infected leaves become necrotic and the growing point is often killed. About this time some of the older leaves develop symptoms which resemble those of virus yellows (Pl. 1, fig. 6). They have a bright yellow intervenal chlorosis, sometimes followed by necrosis, and become thickened and brittle.

The condition can be distinguished from virus yellows, even after obvious signs of fungus attack have disappeared, by the presence of vascular lesions in the veins or petioles. The xylem vessels collapse and become stained dark purplish brown, which colour often shows through the superficial tissues of the stem. The lesion may spread into the lamina, and there affects more superficial tissues which exude a sticky, sugary substance on the under-surface of the leaf (Pl. 1, figs. 6, 7).

When the apical growing point of a plant is killed by the fungus, a number of axillary buds develop. The new leaves remain green, and the outer, yellowed leaves die off. Sometimes these outer leaves rot at the bases of the petioles, and collapse outwards; they may also snap off at their bases, which are very brittle, or they may suffer general necrosis and decay. When they are gone the plant usually appears normal, except for the irregular growth at the centre. If the outer

leaves are still yellow, and do not show the sticky, purplish lesions, the plant probably has virus yellows as well as downy mildew, but it is not always easy to decide by observation alone.

Downy mildew is more closely associated with the seed crops than is virus yellows, but its distribution in relation to the seed crops, and its tendency to spread in rings from isolated foci of infection in the field increase the similarity of its more general characteristics to those of the virus disease.

Deficiency diseases

Magnesium deficiency

Symptoms of magnesium deficiency usually develop during July or August, and persist for the remainder of the growing season. They may be masked by virus yellows and are easily confused with it. The first sign of the disease is the appearance of small patches of pale yellow colour at the upper margins of the leaves. The tissues in these patches enlarge a little, so that the edge of the leaf becomes fluted, or may have a deep fold at the tip. The yellowing spreads inwards and downwards in solid lobes of colour, pushing down between the veins and leaving green healthy tracks between them. The pattern is usually slightly raised by the thickening and enlargement of the affected tissues, and the line of demarcation between green and yellow areas is very well defined (Pl. 2, fig. 10). The surface of the yellowed areas is very smooth and glossy; the yellowed leaves snap easily when broken, but are not so brittle and dry as leaves with virus yellows. The chlorotic areas are usually continuous, but isolated patches may develop between the veins. The colour is not typically as bright as with virus yellows, but after some time it may deepen to a golden yellow.

The yellowing may be followed by necrosis which starts distally and extends down the leaf between the veins. The necrotic tissue is very dark brown and brittle, so that it easily breaks way and the edges of the leaves have a ragged appearance (Pl. 2, fig. 11). If it breaks away from the tip, the leaf becomes truncated and the remaining portion is often green, with little trace of yellowing. A similar truncated leaf may occur with virus yellows, but then the remaining portion of the leaf is nearly always yellow. Sometimes leaves on a plant affected by magnesium deficiency become yellowed uniformly all over, with small scattered necrotic areas (Pl. 2, fig. 8*a*). Very similar symptoms occur in plants with yellows virus, for the scattered 'freckles' of necrosis sometimes run together on a yellowed leaf forming the same kind of patches, and such leaves are almost always pale yellow (Pl. 2, fig. 8*b*).

Typically the symptoms caused by magnesium deficiency are easy to distinguish from those caused by yellows virus. The chlorosis is paler, the surface is glossy, the line of demarcation between yellow and green is better defined, the pattern is slightly raised, and the affected tissues are less brittle. Also the necrosis is darker brown, more frequently confined to the margins of the leaves, and disintegrates much more readily. However, in practice the distinction is not easy to make, especially when the symptoms of both diseases are well advanced. The pattern cannot easily be distinguished when the whole leaf is covered, and necrosis caused by yellows virus may become weathered or blackened by secondary fungus infection. In these circumstances diagnosis requires the help of laboratory techniques.

The habit of magnesium-deficient plants is normal, though they may be stunted, and the necrosis reduces the size and alters the shape of the leaves. The distribution in the field may be patchy, but the patches are generally associated with physical features of the soil, such as drains, depressions or furrows.

Experimental. Table 1 gives the analyses of a number of leaf samples from plants with the symptoms described, compared with leaves from symptomless plants. These samples were taken from widely separated localities at various dates between July and October, in the years 1940–4.

The average composition of healthy sugar-beet leaves at harvest is shown at the foot of the table. These figures are the mean analyses of eight bulked samples from each of eighty-five experiments (1941–4).

The magnesium contents of the affected samples were all below the level for normal beet. The contents of some of the control samples were also low, since each control was taken from the same field as the affected sample. Every plant showing symptoms had a lower magnesium content than its control.

The other elements determined are also all on the average lower in the affected than in the control samples, but the differences are negligible, except for calcium. The mean calcium content of the affected samples is, however, still above normal, while the magnesium is much lower.

Tests were made to assess the accuracy of visual discrimination between symptoms of magnesium deficiency and beet yellows virus. The results are given in Table 2. Six samples of chlorotic leaves were collected from the sugar-beet break of the Rothamsted six-course experiment in 1942 and the symptoms classified by three observers as due to magnesium deficiency or to virus. The leaves of each sample were halved along the midrib, one half being used for serological tests for the virus and the other for spectrographic analysis.

Two samples (nos. 1, 3) which were diagnosed as virus-infected were found by the serological method to contain virus. Two (nos. 5, 6) which were diagnosed as magnesium-deficient proved to have low magnesium contents and gave no virus reaction. One sample (no. 2) judged to be magnesium-deficient was found to contain virus as well as being low in magnesium,

one set of symptoms evidently masking the other. Sample no. 4 which, though chlorotic, was considered typical of neither disease, gave a negative virus reaction and had the highest magnesium content.

Another series of samples was taken on 14 October 1942 from the four parallel strips of plots of the Rothamsted three-course experiment* and from one on the headland beside it. Counts were made of the number of plants showing magnesium deficiency in two rows (about 1000 plants) in each strip. Samples were taken for analysis from three sets of six plants in each strip. The first set was selected for symptoms of beet yellows virus (on the first two strips there were not enough obviously infected plants to supply samples), the second for magnesium deficiency symptoms and the third for freedom from chlorosis. A selection of leaves of all ages was taken from each plant. In Table 3 the leaf analyses are averaged for all the strips.

The samples judged to be magnesium deficient had lower magnesium contents than those judged to be virus-infected, or healthy. The contents of the other elements were normal, or above normal, throughout. The average magnesium content of the virus-infected plants was greater than that of the magnesium-deficient plants, and slightly greater than that of the healthy plants.

Tables 2 and 3 also show some effects of virus on the composition of the leaves. In both, virus considerably decreases the potassium and slightly

TABLE 1. *Analyses of sugar-beet leaves from plants with and without magnesium deficiency symptoms*

	Affected plants					Control plants				
Sample no.	K_2O %	Na_2O %	CaO %	MgO %	Mn p.p.m.	K_2O %	Na_2O %	CaO %	MgO %	Mn p.p.m.
1	1·6	4·2	2·8	0·47	115	1·0	5·1	2·8	0·60	150
2	6·2	2·3	2·3	0·45	85	5·5	2·9	2·2	0·75	63
3	3·4	4·1	1·2	0·16	76	5·3	4·8	1·8	0·35	115
4	2·4	5·1	0·7	0·17	160	3·2	5·2	1·0	0·37	230
5	6·0	1·0	1·2	0·16	62	7·3	1·3	1·9	0·37	51
6	5·0	0·9	1·0	0·13	290	—	—	—	—	—
7	8·0	2·0	2·0	0·16	200	—	—	—	—	—
8	2·0	2·7	1·8	0·28	35	1·3	2·3	2·2	0·36	24
9	2·0	4·0	1·8	0·33	82	2·1	3·8	2·0	0·66	96
10	2·1	2·3	0·8	0·20	84	2·2	2·6	2·1	0·81	250
11	1·7	4·0	1·4	0·22	135	1·1	3·9	2·2	0·39	160
12	3·2	6·0	2·2	0·12	200	2·8	4·6	2·8	0·66	115
13	3·5	4·9	1·1	0·30	25	3·9	5·5	1·8	0·77	47
14	2·7	2·8	1·1	0·34	98	2·9	2·5	1·3	0·41	98
Mean of all samples (14)	3·56	3·31	1·53	0·25	118					
Mean of paired samples (12)	3·07	3·62	1·53	0·27	96	3·22	3·71	2·01	0·54	117
Mean difference (affected − control)	−0·15	−0·09	−0·48	−0·27	−21					
Standard error	±0·24	±0·18	±0·11	±0·05	±17					
Normal sugar-beet leaves						4·16	2·73	1·35	0·65	110

TABLE 2. *Accuracy of visual discrimination between magnesium deficiency and yellows virus symptoms*

Sample no.	Leaf symptoms: Mg deficiency	Leaf symptoms: Virus	Reaction to virus antiserum	Analyses: K_2O %	Na_2O %	CaO %	MgO %	Mn p.p.m.
1	−	++	+	2·5	3·5	1·8	0·38	125
2	+	?	+	2·8	3·0	1·4	0·22	125
3	−	+	+	3·1	2·4	1·1	0·29	72
4	−	−	−	4·0	2·9	2·2	0·47	160
5	++	−	−	4·3	3·3	1·3	0·20	105
6	++	−	−	5·5	1·5	0·7	0·12	45
Mean of virus samples (nos. 1, 2, 3)				2·8	3·0	1·4	0·30	107
Mean of remainder (nos. 4, 5, 6)				4·6	2·6	1·4	0·26	103
Mean of Mg deficient samples (nos. 2, 5, 6)				4·2	2·6	1·1	0·18	92
Mean of remainder (nos. 1, 3, 4)				3·2	2·9	1·7	0·38	119

* This experiment compares various methods of using straw as a manure. Each plot receives the same total amounts of nitrogen, phosphate and potash; none received magnesium at that time.

increases the magnesium content. The effects agree with those previously noted on p. 15. There are no consistent changes in the sodium, calcium and manganese contents.

treatments, arranged in a 16-plot factorial experiment, were as follows:

2½ cwt. magnesium sulphate per acre.
2½ cwt. superphosphate (40 % P_2O_5) per acre.
2 cwt. muriate of potash (60 % K_2O) per acre.
5 cwt. agricultural salt per acre.

All plots received a basal dressing of 4 cwt. per acre sulphate of ammonia.

At Walcot, Lincs. 1943 (Table 5), the same treat-

TABLE 3. *Comparison of leaf analyses of plants showing symptoms of magnesium deficiency and virus yellows*

	Leaf analysis				
	K_2O %	Na_2O %	CaO %	MgO %	Mn p.p.m.
Symptomless (5 samples)	5·0	2·3	2·0	0·49	168
Mg deficiency (5 samples)	5·1	2·3	1·6	0·28	127
Virus (3 samples)	4·2	1·7	1·7	0·51	131
Average sugar-beet leaves	4·2	2·7	1·4	0·65	110

TABLE 4. *Effect of fertilizer treatments on leaf analyses and magnesium deficiency symptoms*

	Leaf analysis					Magnesium deficiency symptoms (score or count)	
	K_2O %	Na_2O %	CaO %	MgO %	Mn p.p.m.		
			Scotter, 1942				
Mean	2·81	5·26	1·30	0·64	42	16	
Effect of Mg	−0·29	0·21	0·12	0·12	14	−17	
Effect of P	0·01	0·14	−0·10	0·02	− 9	− 1	
Effect of K	1·04	−0·44	−0·05	0·03	7	−13	
Effect of Na	−0·49	0·99	0·10	−0·01	2	9	
Standard error	±0·38	±0·61	±0·11	±0·04	± 6	± 5	
			Scotter, 1943				
						16 Aug.	9 Sept.
Mean	3·36	4·10	2·89	0·65	65	212	282
Effect of N	−0·29	0·20	−0·31	0·00	−17	−25	19
Effect of P	−0·05	−0·12	−0·16	−0·03	1	−23	15
Effect of K	1·23	−0·16	0·40	0·14	11	−57	−33
Effect of Na	−0·41	3·27	−0·45	−0·10	17	261	45
Standard error	±0·12	±0·30	±0·23	±0·05	± 6	±67	±44
			Rothamsted, 1942				
Mean	1·94	2·27	2·18	0·36	264	15	
Effect of N	−0·18	0·01	−0·08	−0·02	7	0	
Effect of P	−0·57	0·10	−0·46	−0·01	−44	14	
Effect of K	1·05	−0·30	0·17	−0·04	15	4	
Effect of Na	0·13	0·78	−0·50	−0·06	4	28	
Standard error	±0·11	±0·12	±0·17	±0·02	±23	± 8	
			Bishop Norton, 1944				
Mean	3·61	2·84	1·87	0·60	47	95	
Effect of N+P	−0·12	−0·40	−0·03	0·01	5	13	
Effect of K1	1·20	−0·42	−0·14	−0·08	7	11	
Effect of K2	2·55	−0·11	0·06	−0·03	17	22	
Effect of Na1	−0·24	1·43	−0·17	−0·11	3	93	
Effect of Na2	−0·54	2·21	−0·24	−0·16	5	111	
Standard error	±0·20	±0·19	±0·12	±0·05	± 5	± 9	

N.B. The magnesium figures in Table 4 are greater than those given for magnesium-deficient plants in Tables 1–3. This is because the samples analysed in Table 4 were representative of whole plots and included healthy as well as affected plants.

The effects of magnesium sulphate and other fertilizer treatments on leaf composition and on magnesium deficiency symptoms are shown by data from a number of experiments recorded in Tables 4 and 5. At Scotter, Lincs. in 1942 (Table 4), the

ments were used, but the nitrogen was arranged factorially with the other treatments, making a 32-plot experiment.

The experiments at Scotter in 1943 and Rothamsted in 1942 received no magnesium; the treatments of N, P, K and salt were in a factorial arrangement (see p. 20 for rates of application).

Two levels of both salt and muriate of potash (2½ and 5 cwt. per acre) were used in the experiment at Bishop Norton, Lincs. 1944, and these were arranged factorially with a compound treatment of 4 cwt. sulphate of ammonia per acre and 2½ cwt. superphosphate (40 % P_2O_5) per acre.

No magnesium deficiency symptoms developed on the experiment at Walcot 1943, but on the others the plants showing magnesium deficiency symptoms were counted and random samples of leaves were collected from the plots for analysis. Samples were collected on three dates at intervals from the Walcot 1943 experiment.

TABLE 5. *Effect of fertilizer treatments on percentage* MgO *in sugar-beet leaves at three successive dates*

	% MgO		
Walcot, 1943	21 June	3 Aug.	30 Sept.
Mean	1·18	1·32	1·18
Effect of Mg	0·08	−0·05	−0·02
Effect of N	−0·04	0·04	0·05
Effect of P	−0·01	−0·08	−0·13
Effect of K	−0·04	0·00	0·02
Effect of Na	0·03	−0·10	−0·25
Standard error	±0·03	±0·05	±0·06

In Tables 4 and 5 results are given as effects, the effect of a treatment being the difference in the quantity measured between plots receiving and plots not receiving that treatment.

At Scotter, 1942, magnesium sulphate reduced the symptoms of magnesium deficiency and increased the magnesium content of the leaves. At Walcot (Table 5) magnesium sulphate caused a significant increase in leaf magnesium only in June, but the effect had disappeared in August and September. Salt had caused a big decrease in magnesium content by the last date of sampling. There were no deficiency symptoms on this experiment.

In all the experiments in Table 4 salt increased magnesium deficiency symptoms. At Scotter, 1943, the effect of salt was greatest at the August count, but by September the symptoms had increased more on the no-salt plots and the difference between salt and no-salt plots was consequently much smaller. The effect of potash was much smaller than that of salt, and variable in sign. At Scotter, 1942 and 1943, it reduced symptoms, but at Rothamsted and Bishop Norton it increased them. Where potash reduced symptoms, it increased leaf magnesium, and vice versa. Salt decreased leaf magnesium in every experiment.

The effect of salt on the magnesium content of the leaves is small (averaging −14 %) compared with its effect on symptoms (averaging +96 %), and it seems probable that an increase in the sodium content of the leaves (averaging +81 %) has a direct effect in increasing symptoms.

Nitrogen and phosphate had variable effects on symptoms; none was significant.

Spraying or injecting magnesium sulphate solutions has not been very effective in curing magnesium deficiency symptoms. In a severely affected field at Walcot, Lincs, plots sprayed with a 5 % solution of magnesium sulphate on 11 September 1942 showed no response, and there was also no response to injections with 0·25 % solution.

On 10 July 1943, similar treatments were applied to an affected crop at Norton Disney, Lincs. There was no recovery with four plants injected with 0·25 % $MgSO_4$ solution, but a weak and doubtful response was recorded on three out of four plants injected with 1 % $MgSO_4$ solution. Injections made at the same time on different plants with solutions containing K, Na, Mn, Cu, Fe, N, Zn, Cl, PO_4 ions also had no effects. Other plants were injected on 27 July with 0·25, 1, 2, 5, 10 % solutions of $MgSO_4$. On three plants the leaves above and on either side of the petioles injected with 10 % solution were free from marginal chlorosis on 13 August, whereas the corresponding leaves on the other side of the plants remained chlorotic. Although this suggests a positive result, it was not the clear-cut 'half-leaf' reaction sometimes given by this test with other deficiencies. There was a suggestion of a toxic effect on growth, as the halves of the leaves further from the injected petiole had grown more than the nearer halves, causing the leaves to curve towards the point of injection. The plants injected with the solutions of 5 % and lower concentrations showed no obvious responses.

Plots were sprayed with the same concentrations of magnesium sulphate solution on 27 July. By 13 August there was a general decrease in the intensity of symptoms on both control and sprayed plots, but the growing leaves on the sprayed plots appeared to be slightly greener and to have less marginal chlorosis than on the untreated controls. When examined in September there was no difference to be seen.

Analysis of leaf samples from both sprayed and injected plants failed to show any direct relationship between the magnesium sulphate treatment and the magnesium content. The values varied from 0·20 to 0·31 % MgO in the sprayed series and from 0·14 to 0·66 % in the injected series, but the variations bore no relationship to the concentrations of magnesium sulphate applied.

Although the chlorotic symptoms attributed to

magnesium deficiency are always associated with a low magnesium content of the leaves, there is little experimental evidence to show that the application of magnesium salts will prevent their occurrence. Sprayings and injections gave indefinite results, and the only experiment in which soil applications of magnesium sulphate were made (Scotter, 1942), showed few affected plants, though the number of these was significantly reduced.

It is possible that the symptoms are the result of several interacting factors, not simply of a shortage of magnesium. It has been shown that symptoms are increased by salt and that low leaf magnesium is accompanied by a decreased calcium content (Table 1). It may be that it is necessary to supply calcium as well as magnesium in order to cure the symptoms.

Potash deficiency

The symptoms described below are those commonly attributed to potash deficiency. The authors shared this view at the outset, but observations on various field experiments and analyses of leaf samples have caused them to modify to some extent their ideas of the origin of these symptoms.

The disease is usually referred to as 'scorch', or 'drab-disease', because the principal symptoms are apical and marginal necrosis with a dull olive green appearance of the leaves. The symptoms generally start in July, but in severe cases they may occur earlier. There is first a bronzing and dulling of the surface of the leaf, and later small clusters of diffuse, buff-coloured spots appear, generally arranged in the form of rough triangles with the bases towards the margins of the leaf, and the apices projecting inwards between the veins (Pl. 2, fig. 9). On some plants there is a continuous band of shrunken, chlorotic tissue round the margin of the leaf, and the plants are stunted and wizened-looking. The 'scorch' symptoms develop after the chlorosis or may develop independently of it. The necrosis generally forms an unbroken border round the leaf (Pl. 2, fig. 12), with projecting lobes between the veins. The necrotic tissue is dull or reddish brown and papery in texture. It is rather tough and soft to handle; it does not crumble or disintegrate like the necrosis associated with magnesium deficiency. All the leaves on affected plants are thin and often flaccid and dull-surfaced, giving the characteristic 'drab' appearance to the crop, which is very different from the bright 'contrasty' appearance of crops affected with yellows virus or magnesium deficiency at the same time of the year.

The plant is normal in habit above ground, but tap root development is very poor. Plants affected early in the season are stunted, but often the symptoms do not occur until considerable growth has been made. The symptoms frequently affect 'bolters' more severely than the other plants. The distribution in the field is not usually very patchy, though it may be worse in one part of the crop than in another. Usually if there are severe symptoms on any plants, the rest of the crop will show some sign of it in general dullness and dark colour.

Experimental. Table 6 shows typical analyses of leaves from plants with these symptoms, compared with controls taken from the same fields. These samples were taken from widely separated localities, at various dates between July and December, in the years 1941–4.

In all the examples given in Table 6 the affected plants had lower potassium contents than the controls. Some of the healthy samples also had a low potassium content, but this is because the healthy and deficient samples were from the same field. The sodium content of most of the affected samples was also below the average for normal beet. The affected plants were on the average lower in sodium as well as in potassium, but higher in calcium and magnesium than the controls, all these differences being significant. The approximate limiting value for the appearance of potash deficiency symptoms seems to be 1·0 % K_2O.

The disease has been studied on fertilizer experiments carried out by Rothamsted in conjunction with the sugar-beet factories. These experiments tested the effects of the following fertilizer treatments in all combinations:

0·8 cwt. N per acre as sulphate of ammonia.
1·2 cwt. P_2O_5 per acre as superphosphate.
1·2 cwt. K_2O per acre as muriate of potash.
5 cwt. agricultural salt per acre.

The experiment at Bishop Norton, 1944, is described on p. 19.

Counts of diseased plants or eye estimates of the intensity of symptoms were made on the plots, and random leaf samples were analysed.

Table 7 gives the effects of fertilizer treatments on potash deficiency symptoms and on the mineral composition of the leaves.

Sulphate of ammonia greatly increased symptoms in every experiment and phosphate had on the average a similar but smaller effect. Sulphate of ammonia decreased the potassium, but generally increased the sodium percentage of the leaves; phosphate decreased both. The effects on leaf composition are rather small compared with the increases in symptoms, and it seems that with higher levels of nitrogen and phosphate the plant requires higher contents of potassium and sodium.

In all the experiments, except one, potash reduced symptoms and in all of them it increased the potassium percentage of the leaves. Salt, however, also reduced symptoms, and increased the sodium, but not the potassium content, in all experiments.

At Moulton and Metheringham potash and salt together were necessary to cure the symptoms completely. At Moulton either fertilizer in the absence of the other reduced symptoms very considerably,

but at Metheringham salt was more effective than potash. At Scotter, 1943, and at Bishop Norton, symptoms were completely suppressed by salt alone.

The Cottam experiment was remarkable in that while salt caused a very large reduction in symptoms, potash had no effect, although it increased the potassium content of the leaves considerably. This experiment, however, gave the highest mean potassium content of the whole set and is probably a case of sodium deficiency only.

In general, salt was more effective than potash in reducing symptoms, but it must be remembered that the Na_2O supplied per acre by the salt was rather more than twice the K_2O supplied by the muriate of potash. In the Bishop Norton experiment, however, salt and potash were given at equal rates and at two levels, at both of which salt was more effective than potash in reducing symptoms.

The Whisby experiment was not designed as a potash deficiency experiment, but gave similar results for salt, namely a large reduction in potash deficiency symptoms accompanied by a large increase in the sodium content of the leaves.

Salt does not increase the potassium content of the leaves in any of the experiments, but invariably reduces it, and cannot therefore have cured the symptoms by this means.

The effect of nitrogen and phosphate in increasing potash deficiency symptoms is also shown in the results of observations made in a field at Snitterby, Lincs. Part of the field was laid down to a fertilizer experiment involving dressings of 0–15 cwt. per acre of a compound fertilizer containing 7·6 % N (partly as nitrate of soda), 7·0 % K_2O and 6·7 % P_2O_5. The rest of the field received a fertilizer containing 17·9 % N and 17·9 % P_2O_5, applied at a heavy rate. The plants on the non-experimental part of the field were very rank and showed severe potash deficiency symptoms, especially on 'bolters'. On the experimental plots tops were not so large as those on the non-experimental part of the field, even where the heaviest rates of compound fertilizer were applied. There were no symptoms of potash deficiency on any of these plots.

Samples of leaves were collected from some of the experimental plots, and others from the remainder of the field, where affected and unaffected plants and 'bolters' were sampled separately. The results of the analyses are given in Table 8.

Both potassium and sodium were low on the non-experimental part of the field, where leaf growth was greatly increased by the heavy dressing of nitrogen and phosphate. The bolted plants showed the most severe symptoms and had the lowest sodium and potassium contents. On the experiment, where the nitrogen and phosphate in the fertilizer was balanced by potassium and sodium, the potassium and sodium content of the leaves was higher than in the main crop and no disease symptoms occurred.

Further information on the response of plants showing potash deficiency symptoms to applications of salt and potash were obtained from observations and analyses of samples from a field at Elkesley, Notts. Severe symptoms of potash deficiency were observed in this crop on 23 July 1943. Analyses of leaf samples showed that the plants were low in both potash and sodium (Table 9).

Duplicate plots were treated on 11 August either by broadcasting 5 cwt. of agricultural salt or 1 cwt.

TABLE 6. *Analyses of sugar-beet leaves from plants with and without potash deficiency symptoms*

	Affected plants					Control plants				
Sample no.	K_2O %	Na_2O %	CaO %	MgO %	Mn p.p.m.	K_2O %	Na_2O %	CaO %	MgO %	Mn p.p.m.
1	0·89	1·8	2·0	2·9	95	1·87	2·0	1·5	0·8	51
2	0·94	1·0	2·3	2·3	390	3·67	1·4	2·0	2·3	290
3	0·80	2·7	2·5	1·1	84	1·13	2·9	2·2	0·9	54
4	0·64	0·8	2·5	1·3	870	4·45	1·8	1·6	0·7	230
5	0·64	0·8	3·5	1·6	155	2·16	2·6	2·1	0·8	250
6	1·18	1·3	3·2	1·9	105	4·04	1·1	2·4	1·9	220
7	0·52	2·0	3·5	1·0	175	1·62	3·5	1·4	0·6	145
8	1·05	1·4	2·0	3·7	160	1·96	1·1	2·0	3·5	320
9	0·35	1·6	3·6	0·9	890	1·16	3·4	1·4	1·2	560
10	0·73	4·0	4·0	1·5	23	0·88	3·3	2·8	1·2	50
11	0·88	2·5	3·9	2·1	860	2·16	2·7	2·1	1·3	440
12	0·87	2·9	1·8	0·9	22	1·06	3·2	1·9	0·9	20
13	0·41	0·8	3·8	1·4	69	1·32	1·0	2·2	1·2	42
14	0·37	2·5	1·9	0·8	83	0·98	3·2	1·4	0·7	110
Mean of all samples (14)	0·73	1·86	2·89	1·67	284	2·03	2·37	1·93	1·29	199
Mean difference (affected − control)	−1·30	−0·51	0·96	0·38	85					
Standard error	±0·29	±0·20	±0·20	±0·16	±60					
Normal sugar-beet leaves						4·16	2·73	1·35	0·65	110

TABLE 7. *Effect of fertilizer treatments on leaf analyses and potash deficiency symptoms*

	Leaf analysis					Potash deficiency symptoms (score or count)	
	K_2O %	Na_2O %	CaO %	MgO %	Mn p.p.m.		
Moulton, 1942							
Mean	2·71	2·45	1·22	0·82	169	12	
Effect of N	−0·85	0·07	−0·14	0·01	18	12	
Effect of K	1·03	−0·28	−0·02	0·02	6	− 20	
Effect of Na	−0·19	1·81	−0·17	−0·10	55	− 20	
Metheringham, 1942							
Mean	3·86	2·70	2·92	1·62	54	10	
Effect of N	−0·68	0·08	0·19	0·21	29	9	
Effect of P	−0·72	−0·30	−0·24	−0·44	20	11	
Effect of K	1·42	0·20	−0·04	0·09	9	− 5	
Effect of Na	−0·62	3·02	−0·76	−0·29	11	− 15	
Standard error	±0·38	±0·13	±0·12	±0·07	± 7	± 3	
Cottam, 1943							
Mean	4·99	3·36	1·28	1·17	454	92	
Effect of N	−0·96	0·60	−0·25	−0·09	328	179	
Effect of P	−0·37	−0·11	−0·01	−0·04	13	− 11	
Effect of K	−1·21	−0·51	0·08	−0·01	219	3	
Effect of Na	−0·72	2·67	0·18	0·02	21	−165	
Standard error	±0·11	±0·30	±0·14	±0·08	±96	± 42	
Scotter, 1943						16 Aug.	9 Sept.
Mean	3·36	4·10	2·89	0·65	65	38	115
Effect of N	−0·29	0·20	−0·31	0·00	−17	76	198
Effect of P	−0·05	−0·12	−0·16	−0·03	1	34	78
Effect of K	1·23	−0·16	0·40	0·14	11	−20	− 48
Effect of Na	−0·41	3·27	−0·45	−0·10	17	−76	−230
Standard error	±0·12	±0·30	±0·23	±0·05	± 6	±14	± 60
Bishop Norton, 1944							
Mean	3·61	2·84	1·87	0·60	47	73	
Effect of N+P	−0·12	−0·40	−0·03	0·01	5	133	
Effect of K1	1·20	−0·42	−0·14	−0·08	7	− 30	
Effect of K2	2·55	−0·11	0·06	−0·03	17	− 91	
Effect of Na1	−0·24	1·43	−0·17	−0·11	3	−145	
Effect of Na2	−0·54	2·21	−0·24	−0·16	5	−177	
Standard error	±0·20	±0·19	±0·12	±0·05	± 5	± 26	
Whisby, 1944							
Mean	1·80	5·66	2·49	1·70	194	449	
Effect of N	−0·05	−0·28	0·01	0·04	46	78	
Effect of Na	−0·04	5·38	−0·64	−0·22	61	−834	
Standard error	±0·06	±0·24	±0·13	±0·08	±11	± 81	

N.B. The potash figures in Table 7 are considerably greater than those given for potash-deficient plants in Table 6. This is because the samples analysed in Table 7 were representative of whole plots and included healthy as well as affected plants.

muriate of potash per acre, or by spraying the plants with 1 cwt. per acre of agricultural salt in solution.

Petiole injections were made on other typically affected plants by Roach's method on 11 August, with various concentrations of KCl and NaCl. Three plants were used for each concentration.

On 18 September, leaf samples were collected from the treated plots and were examined for symptoms and then analysed. Leaves from around the injected petioles were dealt with similarly. The results are given in Table 9.

The potash and salt treated plots all improved in size and colour compared with the untreated parts of the field, although some symptoms of potash deficiency still remained. The analyses showed that the potassium content was slightly, and the sodium content greatly, increased by applications of the respective fertilizers.

On the injected plants there was a slight improvement in growth and colour of the halves of the leaves nearest to the injected petioles when solutions between 0·25 and 2 % of salt and potash were injected. Higher concentrations had a toxic effect and the leaves nearest to the injected petioles died. The analyses were made on whole leaves of which only halves had shown improvement, and may underestimate the change in mineral content associated with the improvement. The analyses show increasing sodium contents for increasing concentrations of salt injected, and increasing potassium contents for solutions over 2·0 %.

The experimental results given in this section show

TABLE 8. *Analyses of leaves from Snitterby, Lincs*, 1941

Manuring	Type of leaf	Leaf analysis K_2O %	Leaf analysis Na_2O %
	Main crop		
N and P compound:			
Heavy dressing	Healthy leaves	3·1	1·2
	Diseased leaves	0·89	1·04
	Diseased 'bolters'	0·40	0·60
	Experimental area		
N, P, K and Na compound:			
15 cwt. per acre	Healthy leaves	4·4	2·1
6 cwt. per acre	Healthy leaves	5·4	2·1
No fertilizer	Healthy leaves	5·0	2·0

TABLE 9. *Analyses of leaves from Elkesley, Notts*, 1943

	Proportion of leaves with symptoms	Leaf analysis K_2O %	Leaf analysis Na_2O %
	General sample		
Healthy leaves	None	1·96	1·12
Affected leaves	All	1·05	1·42
	Treated plots		
1 cwt. per acre muriate of potash broadcast	27/96	1·93	1·9
5 cwt. per acre agricultural salt broadcast	7/72	1·11	6·0
1 cwt. per acre agricultural salt sprayed	15/87	0·97	4·0
Untreated	21/85	1·30	2·3
	Injected plants		
*Untreated	12/35	0·87	1·51
0·25 % KCl solution	6/12	0·77	1·41
1·0 % KCl solution	5/12	0·62	1·40
2·0 % KCl solution	Toxic (?)	1·20	1·60
5·0 % KCl solution	Toxic	3·1	1·09
10·0 % KCl solution	Toxic	5·1	1·56
0·25 % NaCl solution	4/12	0·86	1·30
1·0 % NaCl solution	3/9	0·67	1·79
2·0 % NaCl solution	2/12	0·84	2·30
5·0 % NaCl solution	Toxic	0·67	4·0
†10·0 % NaCl solution	Toxic	0·68	5·5

* Untreated samples are from nearby plants which had not been injected.

† The leaves adjacent to the injected petioles were killed, so the nearest remaining were analysed.

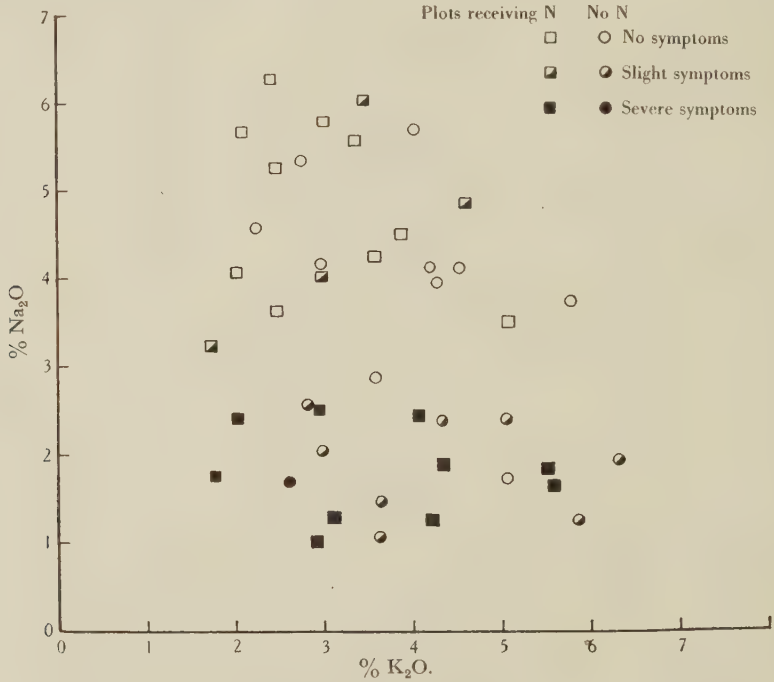
that the symptoms which have been described as potash deficiency can occur not only when the potassium content of the plants is low but also when the sodium content is low, even though the potassium is high. Symptoms are induced by dressings of nitrogen or phosphate in the absence of potash and salt.

Text-fig. 1 shows the distribution of samples from the experiments in Table 7 (8 per experiment) with severe, slight or no symptoms of potash deficiency. Percentage K_2O in the leaves is plotted against % Na_2O and plots receiving sulphate of ammonia are distinguished from those receiving none. Severe symptoms occur mainly on plots receiving nitrogen. These all lie in the region of lower values of % Na_2O, but cover a wide range of potassium percentages, from 2 to 6 % K_2O.

The general conclusion is that the sugar-beet plant can suffer from sodium deficiency and this may be accompanied by some degree of potash deficiency.

It is necessary also to postulate some interchangeability in the functions of sodium and potassium in the plant. If the two deficiencies were entirely separate disorders which happen to cause indistinguishable symptoms, it would be necessary to supply both potash and salt in order to obtain any reduction of symptoms. In fact, as at Metheringham, cases occur in which symptoms are substantially reduced by either of the two fertilizers applied separately.

It might be argued that salt merely alleviates the outward signs of potash deficiency, but leaves the plant still suffering from lack of potassium. As well as reducing symptoms, salt in fact causes a greater increase in crop dry matter per acre than does potash in every experiment quoted for which yield figures are available. This must mean that an actual shortage of sodium is being made good.



Text-fig. 1. Incidence of potash deficiency symptoms observed on the fertilizer experiments, related to the potassium and sodium contents of the leaves. Each point is the mean of four plots (two phosphate treatments, two replications).

Manganese deficiency

Manganese deficiency, commonly known as 'speckled yellows', is usually easy to distinguish from other chlorotic diseases of sugar beet. The symptoms may develop in May or June, which is earlier than other diseases causing chlorosis are normally found in the field. In severe cases the growth of the plants is checked. Badly affected fields can be recognized from a distance by their pale, greenish-yellow colour and by the 'staring' effect produced by the upright habit of the plants and the pointed shape of the leaf caused by the in-curling of the margins. The symptoms decrease in intensity in August, and by October the plants may appear to have made a complete recovery.

The first symptom of manganese deficiency is a chlorotic mottle in the intervenal areas of the leaf laminae. These lesions spread into a feathery pattern of pale whitish green which eventually occupies most of the spaces between the veins (Pl. 2, fig. 13). The texture is dry and papery and the margins of the leaf curl inwards. The chlorotic areas develop small, angular, necrotic patches which are pinkish-buff, translucent and of horny texture (Pl. 2, fig. 14). These sometimes drop out leaving small, angular holes in the leaves, which can be seen, after the recovery of the plant, surrounded by healthy green leaf tissue. The young leaves of badly affected plants are stunted and deformed by lack of growth of the chlorotic intervenal areas and curling of the margins. When the plants are recovering, the pattern becomes more diffuse as the pale lesions gradually return to normal green. In this transition stage the disease is less easy to distinguish from other chlorotic diseases.

The fertilizer experiment at Metheringham in 1942 mentioned on p. 20 showed symptoms of manganese as well as potash deficiency. The plots were scored for manganese deficiency symptoms on 24 July, and leaf samples were taken for analysis. The effect of the treatments on the manganese in the leaves and on the symptoms are given in Table 11.

Sulphate of ammonia, potash and salt decreased the symptoms and increased the manganese content of the leaves. Phosphate had no effect on symptoms, although it increased leaf manganese.

The effect of sulphate of ammonia, potash and salt in increasing the manganese content of sugar-beet leaves is confirmed by the analyses of samples from other experiments of the same design as that at Metheringham, carried out in 1941. Table 11 shows also the average treatment effects for six of these experiments, in which the crop had a low manganese content comparable with that at Metheringham.

TABLE 10. *Analyses of sugar-beet leaves from plants with and without manganese deficiency symptoms*

	Affected plants					Control plants				
Sample no.	K_2O %	Na_2O %	CaO %	MgO %	Mn p.p.m.	K_2O %	Na_2O %	CaO %	MgO %	Mn p.p.m.
1	1·3	5·2	1·7	0·6	6	2·8	4·5	2·5	0·9	20
2	7·4	3·8	2·5	1·6	30	7·0	5·3	2·5	2·8	115
3	8·8	3·4	2·2	0·8	5	8·2	4·0	2·3	1·1	9
4	1·1	6·7	2·0	1·7	9	1·4	8·5	1·6	0·7	93
5	3·1	5·1	2·1	0·8	6	2·5	4·6	1·8	0·7	23
6	3·4	7·7	1·4	0·9	5	1·4	7·1	1·2	0·6	7
7	4·4	2·9	1·0	0·6	11	3·4	2·1	1·1	0·7	19
8	3·8	5·5	1·3	0·5	15	3·9	5·5	1·8	0·8	47
9	0·7	4·0	4·0	1·5	23	0·9	3·3	2·8	1·2	50
10	1·8	3·3	1·7	0·6	10	2·5	1·8	1·1	0·4	10
Mean of all samples (10)	3·58	4·76	1·99	0·96	12	3·40	4·67	1·87	0·99	39
Mean difference (affected − control)	0·18	0·09	0·12	−0·03	−27					
Standard error	±0·31	±0·34	±0·18	±0·18	±10					
Normal sugar-beet leaves						4·16	2·73	1·35	0·65	110

It occurs particularly on fen and marsh soils, light sands and gravels, and on newly ploughed grassland. It is accentuated by alkaline soil conditions. Individual plants vary considerably in their susceptibility to manganese-deficient conditions; one plant may show pronounced symptoms while the neighbouring plants appear to be quite healthy.

Experimental. Table 10 gives analyses of leaves from plants showing 'speckled yellows' symptoms compared with controls taken from the same field and in some cases from neighbouring plants.

All the affected plants are lower in manganese than are their controls, but some of the healthy looking plants collected as controls also had very low manganese content, and all were below the average manganese content for normal sugar beet. Plants showing symptoms almost invariably have a manganese content of less than 25–30 parts per million, but the reverse is not necessarily true.

As at Metheringham, sulphate of ammonia and salt have on the average greater effects in increasing the manganese content than has potash. No figures are available in these experiments for the effect of phosphate.

An experiment was carried out in 1943 to show the effect of spraying a crop of sugar beet, which was showing 'speckled yellows' symptoms, with manganese sulphate solutions. The manganese sulphate was applied at five rates, viz. 0, 5, 10, 20, 40 lb. per acre. The crop was sprayed on 5 July and leaf samples were taken at three subsequent dates, 15 July, 3 August and 30 September. The plots were also scored for deficiency symptoms on 3 August. Table 12 gives leaf analyses for manganese and deficiency symptom scores for the five treatments.

There was a decrease in symptoms with increasing rate of application of manganese, accompanied by an increase in the manganese content of the leaves at

the same date, 3 August. Comparing the leaf analyses on the three sampling dates, it is noticeable that the effect of the treatments on the manganese contents diminishes considerably with time. This is probably because the manganese is immobilized in the sprayed leaves and steadily lost to the plant by the death of these leaves.

Symptoms of manganese deficiency decrease or disappear very rapidly when petioles are injected with solutions of manganese sulphate. Six badly affected plants were injected with a 0·25 % solution of $MnSO_4$ on 20 August 1942; by 25 August, the halves of the leaves nearest to the injected petiole were decidedly greener than the rest of the leaves. The chlorotic symptoms on these halves of the leaves had almost disappeared by 27 August, and on 2 September there was a pronounced growth effect, i.e. the injected halves of the youngest leaves had grown more than the halves away from the site of the injection and the mid-ribs were curved. The growth effect persisted until the plants were harvested in October, when the sector of the plants around the site of the injection was larger than the remainder.

Similar results were obtained in 1943; these are given in Table 13.

Injections were made with a range of concentrations of manganese sulphate. Concentrations of 0·25 and 0·5 % $MnSO_4$ cured the symptoms, but higher concentrations proved toxic. Analysis of leaves from around the injected petioles shows that large amounts of manganese were taken up with the higher concentrations (cf. below, Manganese excess).

These experiments confirm that the symptoms of 'speckled yellows' are due to deficiency of manganese in the leaves. The level of manganese which can produce these symptoms varies with individual plants and growth conditions. The symptoms are easily cured by application of manganese sulphate and may be reduced, and the manganese content of the leaves increased, by sulphate of ammonia, potash and salt applications.

TABLE 11. *Effect of fertilizer treatments on leaf manganese and manganese deficiency symptoms*

	Metheringham, 1942		Mean of 6 exps. in 1941
	Manganese deficiency score	Leaf analysis Mn p.p.m.	Leaf analysis Mn p.p.m.
Mean	9	54	52
Effect of N	−12	29	13
Effect of K	−10	9	6
Effect of Na	−10	11	15
Effect of P	2	20	—
Standard error	±4	±7	—

TABLE 12. *Manganese spraying experiment at Walcot*, 1943

Treatments applied 5 July as spray	Leaf analyses—p.p.m. Mn			Mn deficiency scores
	15 July	3 Aug.	30 Sept.	3 Aug.
A. Control	20	10	16	39
B. 5 lb. $MnSO_4$ per acre	105	21	19	21
C. 10 lb. $MnSO_4$ per acre	172	29	20	19
D. 20 lb. $MnSO_4$ per acre	350	55	20	17
E. 40 lb. $MnSO_4$ per acre	560	79	26	14

TABLE 13. *Effect of manganese sulphate injections of plants affected with 'speckled yellows', Walcot* 1943

Solutions injected 5 July	Effect on leaves		Analysis of leaves collected 3 Aug. p.p.m. Mn
	14 July	3 Aug.	
0·25 % $MnSO_4$	2/3 response	3/3 response	36
0·5 % $MnSO_4$	1 response: 2 toxic	3/3 response	89
1·0 % $MnSO_4$	3/3 toxic	Toxic (?)	220
2·0 % $MnSO_4$	3/3 toxic	Toxic (chlorotic)	4700
5·0 % $MnSO_4$	3/3 toxic	Toxic (stunted)	3550

HALE, WATSON AND HULL—CAUSES OF CHLOROSIS AND NECROSIS OF SUGAR-BEET FOLIAGE

HALE, WATSON AND HULL—CAUSES OF CHLOROSIS AND NECROSIS OF SUGAR-BEET FOLIAGE

Manganese excess

Another disorder resulting in chlorosis may be mentioned here. It occurs in well-defined patches, in which the centrally-placed plants are worst affected. The plants are severely stunted, and the foliage and leaf stems are uniformly pale yellowish-green without a trace of mottle or speckling, though later in the season a mottle may develop. The plants have an upright appearance and the margins of the leaves roll inwards.

Analysis of the leaves reveals that the manganese contents run into thousands of parts per million, instead of the usual hundreds or less. Table 14 gives analyses for a number of leaf samples taken from several widely separated localities at various dates between July and November in 1942 and 1943.

Two of the control samples are also very high in manganese, but the corresponding affected samples are even higher. There is no deficiency among the other elements determined; in fact potassium and magnesium are considerably above normal.

The affected plants occur in patches where the soil is acid, with a *p*H generally below 5. The acidity has not resulted in calcium deficiency, for the calcium contents are on the average above normal, but appears to have affected the plants by raising the manganese contents to excessive values.

TABLE 14. *Analyses of leaves from plants with and without symptoms of manganese excess*

	Affected plants					Control plants				
Sample no.	K_2O %	Na_2O %	CaO %	MgO %	Mn p.p.m.	K_2O %	Na_2O %	CaO %	MgO %	Mn p.p.m.
1	8·7	1·5	2·8	1·8	1560	9·6	1·3	2·4	1·8	178
2	2·1	2·3	1·1	0·8	1720	—	—	—	—	—
3	8·7	2·7	2·2	1·9	2160	8·4	2·2	2·6	1·6	113
4	3·6	3·7	2·5	1·5	2690	5·3	3·2	2·6	1·9	1490
5	7·5	1·6	2·0	1·2	1250	—	—	—	—	—
6	6·3	2·8	2·0	2·0	2290	—	—	—	—	—
7	5·5	2·9	3·5	0·8	3020	9·3	1·9	2·1	1·0	1700
Average sugar-beet leaves						4·16	2·73	1·35	0·65	110

Our grateful thanks are due to Mrs E. J. Bradley and Dr C. E. Cornford for assistance with field observations and sampling, and to the agricultural staffs of the Bardney, Brigg, Kelham and Peterborough factories of the British Sugar Corporation for their valuable co-operation in the experiments. The photographs were taken by V. Stansfield.

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EXPLANATION OF PLATES 1 AND 2

PLATE 1

Fig. 1. Leaf from plant infected with beet mosaic virus showing 'vein-band' symptoms (photographed by transmitted light).

Fig. 2. Leaf from plant infected with beet mosaic virus showing 'ringspot' symptoms (photographed by transmitted light).

Fig. 3. Leaf from plant infected with beet yellows virus showing necrotic symptoms (early infection).

Fig. 4. Leaf from plant infected with beet yellows virus showing scarlet 'freckles' (Kleinwanzleben 'E'). Late infection.

Fig. 5. Leaves from plants infected with beet yellows virus (late infection); (*a*) angular patch of chlorosis bounded by veins and placed asymmetrically on lamina; (*b*) apical patch.

Fig. 6. Leaf from plant infected with downy mildew showing diffuse intervenal chlorosis.

Fig. 7. Leaf from plant infected with downy mildew showing chlorosis and necrosis spreading from vascular lesions.

PLATE 2

Fig. 8. (*a*) Leaf from plant with magnesium deficiency showing uniform chlorosis with isolated necrotic lesions; (*b*) similar leaf from plant with virus yellows, but note fine necrotic 'freckles' over surface of leaf.

Fig. 9. Leaf from plant showing early 'potash deficiency' symptoms.

Fig. 10. Typical symptoms of magnesium deficiency with well-defined chlorotic lobes.

Fig. 11. Necrosis associated with magnesium deficiency, note 'frizzled' appearance of leaf margins.

Fig. 12. Advanced 'potash deficiency' with almost complete necrosis.

Fig. 13. Chlorosis caused by manganese deficiency.

Fig. 14. Necrosis caused by manganese deficiency. In parts of the leaf the chlorosis is fading as the plant is recovering from the most severe symptom, but the necrosis remains as small isolated lesions.

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Verticillium wilt of sainfoin*

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(With Plate 3 and 3 Text-figures)

A wilt disease of sainfoin caused by *Verticillium Dahliae* Kleb. is described, and it is shown that the fungus can penetrate sainfoin seedlings through unwounded roots as well as through ruptures caused by the emergence of lateral rootlets. *V. Dahliae* was isolated from naturally infected soil only in June, July and August, although another species, *V. nigrescens*, was obtained throughout the year.

Comparative studies of the longevity of cultures of *V. Dahliae*, *V. albo-atrum* and *V. nigrescens* proved that all are viable for at least 3 years on agar media. On sterilized wheat grains *V. Dahliae* dies within 8 weeks after inoculation, *V. albo-atrum* and *V. nigrescens* within 12 weeks, while the hyaline variants of the first two remain viable for 6 months.

Evidence was obtained that in artificially inoculated soil *V. Dahliae* persists mainly as microsclerotia. The fungus may also exist in the soil as hyaline mycelium or conidia, but only for a relatively short time.

The incidence of this disease in sainfoin is reduced by an increase in soil-water content, but is unaffected by the application of lime to the soil.

In July 1940 a microsclerotia-forming species of *Verticillium*, *V. Dahliae*,† was isolated by Prof. F. T. Brooks from wilting sainfoin plants (common and giant) growing at the Plant Breeding Institute, University Farm, Cambridge. This appears to be the first record of the disease in Britain, but on the Continent Richter & Klinkowski (1938) had reported an attack, ascribed to *V. albo-atrum*, on this host and on lucerne in Germany. The original outbreak at Cambridge appeared to be restricted to a plot at the Plant Breeding Institute (infected site 1), since no diseased plants could be discovered on a sainfoin ley 400 yd. away. Naturally infected plants were subsequently found in a trial at the Field Station of the Botany School, Cambridge (site 2), and on a plot at the National Institute of Agricultural Botany, Cambridge (site 3). No wilt, however, has yet been seen on any of the numerous sainfoin leys examined in East Anglia.

* Part of a Thesis for the degree of Ph.D. in the University of Cambridge.

† In this paper the form of *Verticillium* producing microsclerotia and that producing black carbonized resting mycelium are referred to the species *V. Dahliae* and *V. albo-atrum* respectively.

SYMPTOMS

The leaflets of the outer leaves of affected plants first become pale green and fold upwards along the mid-rib, then turn yellow and finally brown as they wither and die. Later, the inner leaves develop similar symptoms, and during the hot summer months, when the disease progresses most rapidly, these may show 'true wilt', i.e. a collapsed condition due to a loss of cell turgor (Pl. 3, fig. 1). Apart from the general wilting no external signs of the disease develop on the stems or roots of infected plants. Richter & Klinkowski (1938) stated that affected sainfoin plants when completely dead were covered by the whorled conidiophores characteristic of *Verticillium*, but this was observed by the writer only on dead leaflets previously placed in a moist chamber.

At site 1 plants which appeared dead during the winter occasionally threw out new green shoots in the

following spring, but these were weak and useless. This capacity of the sainfoin plant for partial recovery corresponds to reports by investigators of other plants infected by *Verticillium*, e.g. Harris (1925, 1936), Van der Meer (1925) and Rudolph (1931).

Cut stems and roots of affected plants show the dark brown discoloration of the wood characteristic of *Verticillium* hadromycosis (Pl. 3, fig. 2). In a completely wilted plant this discoloration can be traced from the small lateral rootlets through the main root up into the stems, occasionally extending into the leaf petioles. It can be shown that the fungus also invades the leaflets, since conidiophores grow out when dead leaflets from wilted plants are placed in a moist chamber.

Fungal invasion is confined to the wood cylinder, even after the death of the plant, and the amount of mycelium in the vessels may vary from a few hyphae to a dense bundle. Brownish gum inclusions are also often present, but no spores or microsclerotia have been seen within diseased tissue, and tyloses, common in many hosts attacked by this fungus, were never observed.

TABLE 1. *Sainfoin in naturally infected soil*

Year	Living plants (survivors)	No. wilted	Wilted (%)
	Site 1		
1941	80	21	26
1942	59	21	36
1943	38	30	78
	Site 2		
1941	80	16	20
1942	64	22	33
1943	42	22	52

V. Dahliae was isolated from all the wilted plants from both localities.

SEASONAL PROGRESSION OF ATTACK

Serious losses due to this disease had occurred on site 1 during the summer months in several successive years. To assess the degree of attack eighty 3-months old common sainfoin plants (forty Hunter's variety and forty Benson's variety) were planted on 15 May, 1941 at site 1, and a similar number at site 2. Signs of wilt appeared at both sites about 6 weeks later, and the numbers of plants which died during the following three summers are given in Table 1. As no differences in the development of the disease in the two varieties were observed, both are grouped together in the table.

From this table it is seen that the disease spread rapidly at both sites. The slightly greater incidence of disease on site 1 may be explained by the fact that here only sainfoin had been grown for some years, and wilt had been observed prior to the present investigation. This may have resulted in a greater initial concentration of the fungus on site 1 (see also 'Isolation from soil', below).

The earliest date on which the disease was observed in the three years was 21 May in 1942, but in each year severest and most extensive wilt occurred during the hot months of June, July and August. Plants apparently healthy in the morning might completely wilt by the end of the day, but occasionally one showing 'true wilt' during a hot afternoon would partially recover in the cool of the evening.

To determine whether plants raised directly from seed sown *in situ*, i.e. not from transplanted seedlings, were also susceptible to infection by *V. Dahliae*, four rows were sown with common sainfoin seed (two with 'Hunter' and two with 'Benson') on infected site 1 in July 1942. Many of the resulting undisturbed plants developed wilt symptoms during the following summer, showing that the fungus can and does infect such plants.

THE PATHOGEN (*VERTICILLIUM DAHLIAE* KLEB.)

(1) *Establishment of pathogenicity*

Inoculations of both common and giant sainfoin (grown from seed in uninfected soil) from pure cultures of the fungus isolated by Brooks were made by various methods, with results as follows:

(i) *Wounding*

(*a*) *Roots.* A fragment of an agar culture of the fungus was placed in cuts made with a sterilized scalpel in the main root just below ground-level. Five of six common sainfoin plants and all of six giant plants, thus inoculated, started to wilt after 6–12 weeks. The six control plants (wounded only) remained healthy.

(*b*) *Stems.* Sainfoin plants (Hunter) were wound inoculated (as above) at varying places along the stems. Five plants of six inoculated developed wilt symptoms. The three control plants remained healthy.

(ii) *Infection of the soil*

Seedlings were transplanted in soil (in pots) to which a 4-weeks old culture of the fungus on sterilized wheat grains had been added. Six of six 'common' and five of six 'giant' plants wilted after 6–10 weeks. Six controls growing in soil containing uninfected wheat grains remained healthy.

From all the wilted plants in the above experiments *V. Dahliae* was reisolated.

Where the sainfoin plants were grown in infected soil the discoloration of the wood in the main root could be traced down as far as some of the small lateral rootlets, below which the wood appeared normal, suggesting that the actual penetration of the fungus was effected through these rootlets. By sectioning the major roots the xylem vessels just above the lowest discoloured lateral rootlet were found to be clogged with gum and hyphae. At the

level of the rootlet, the vessels of the major root on the side of the rootlet emergence were filled with gum and hyphae, whilst those on the opposite side contained only hyphae, indicating that gum formation follows mycelial invasion.

(2) *Mode of penetration*

It has generally been assumed that *Verticillium* attacks plants through the root system, and most workers conclude that the fungus can penetrate healthy roots, but only Van der Meer (1925) and Dufrénoy (1927) appear to have investigated in any detail the exact mode of infection of healthy tissue by this parasite. Both these investigators observed conical protuberances on the walls of the cells directly opposite the point of penetration of the fungus.

In the present studies the method of Virgin & Walker (1940), in their investigation into near-wilt

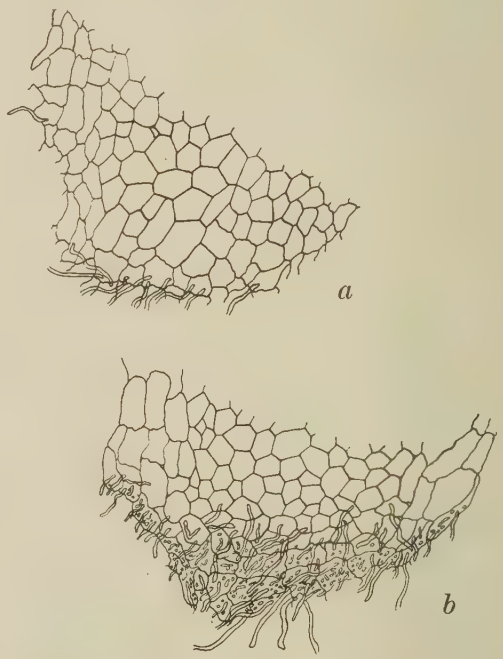
TABLE 2. *Summary of observations*

Interval in days of exposure to infection drop	Observations
1	No infection
2	Very early stages of infection in root cap region with the broken cells penetrated by hyphae (Text-fig. 1)
3	Root hairs and epidermal cells of hypocotyl and young stem penetrated (Text-fig. 2). Mycelium also entering through ruptures in main root caused by the emergence of lateral rootlets (Text-fig. 3)
4	Roots and young stems appeared brown to the naked eye
9	Seedlings deeply penetrated at all points of contact

disease of peas (*Fusarium oxysporum*), was used. Common sainfoin (Hunter) seeds were surface-sterilized by immersion for 5 min. in a 0·1 % solution of mercuric chloride after washing in a solution of soap and water. They were then rinsed in sterile distilled water and incubated at 25° C. upon sterile moistened filter-papers in Petri dishes. Immediately after germination the seedlings were removed to plates of Dox's medium in 9 cm. Petri dishes and a spore suspension of *V. Dahliae* was placed in contact with the seedlings (Pl. 3, fig. 3) in the region of (*a*) the root cap, (*b*) root hairs, (*c*) the zone of emergence of the lateral rootlets, (*d*) the hypocotyl and (*e*) the young stem.

The seedlings were removed at intervals of 24 hr. after inoculation and cut into small pieces which were then fixed in formalin-acetic-alcohol, embedded in wax, microtomed, and the sections stained with Delafield's haematoxylin. Infected seedlings were also stained entire in cotton blue (in lactophenol), and on mounting in lactophenol and crushing under a cover-slip the hyphae could be clearly seen within the root hairs. The results are shown in Table 2.

As shown in Text-figs. 1 and 2 penetration of the cells appeared to be mechanical, no protuberances as recorded by Van der Meer and Dufrénoy being observed. In root-hair penetration the very slight swelling of the hypha in actual contact with the cell wall is of particular interest. A similar swelling was also seen in the cortical region whenever the tip of a hypha was in contact with a cell wall. In infection through the root cap the fungus was observed to grow rapidly through this region into the actively dividing



Text-fig. 1. Penetration of root-cap region of young sainfoin root after 2 days (*a*), and after 5 days (*b*) in contact with *V. Dahliae* on Dox's agar (× 400).

apical cells and so into the initials of the vascular system. Where infection occurred immediately behind the root cap the mycelium spread out fan-wise in the cortex, growing rapidly towards the vascular cylinder which was penetrated through the thin-walled region of the annularly and spirally thickened vessels. From this experiment it is concluded that wounds are not necessary for penetration of the roots of sainfoin by *V. Dahliae*.

SOIL-PATHOGEN RELATIONS

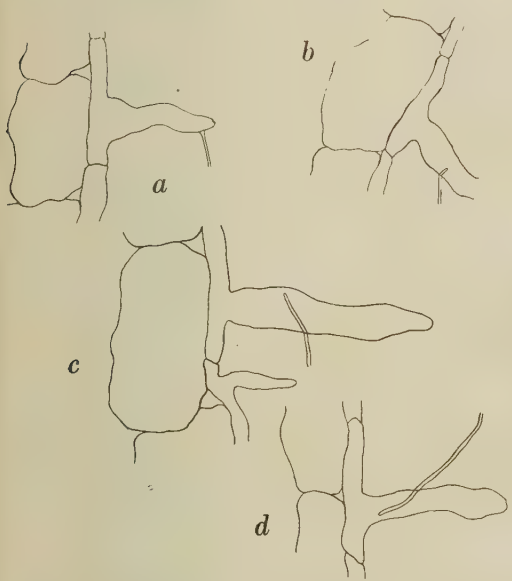
In diseases caused by *Verticillium*, initial infection usually occurs through the root system which, together with the fact that this pathogen can be grown

on artificial media, suggests that it may persist as a saprophyte in the soil, but in what form is not yet definitely known.

(1) *Isolation from soil*

Of the methods tested using artificially infected soil the water dilution method of Brierley *et al.* (1927) proved most successful. For plating, 1 c.c. of a 1:10,000 or 1:20,000 dilution was added to 15 c.c. of medium. Using this method monthly attempts were made to isolate *Verticillium* from the naturally infected soil of site 1, the samples being taken from the top 6–8 in. in the vicinity of diseased plants, with the following results:

V. nigrescens Peth., a chlamydospore-forming species, was isolated every month of the year.



Text-fig. 2. Penetration of root hairs of young sainfoin after 2 days (*a*), 3 days (*b*), 5 days (*c*) and 6 days (*d*) in contact with *V. Dahliae* on Dox's agar (× 400).

V. Dahliae, however, only appeared on the plants during June, July and August, and even in these months negative results were obtained from soil 1 ft. or more from diseased plants. It appears, therefore, that during the summer months a concentration of *V. Dahliae* is built up near affected plants, but whether this concentration is the primary cause of infection or the result of the fungus growing out from the wilting plants is uncertain. In field observations it was noticed that the disease tended to spread out from the first infected plant to its neighbours, suggesting that infection spreads either by root contact or by the roots of neighbouring plants growing into heavily contaminated soil in the region of the first infected plant.

(2) *Viability on culture media*

Agar media. *V. Dahliae*, *V. albo-atrum* and *V. nigrescens* were all found to be viable after 3 years on test-tube slants of Dox's or prune-extract agars.

Sterilized wheat grains. All soil inoculations were made by mixing wheat-grain cultures of *Verticillium* with the soil. Before making these inoculations, however, the length of time the fungus remained viable on this medium was determined. Forty grammes of wheat grains in 150 c.c. of distilled water were autoclaved in a 500 c.c. flask for 20 min. at 120° C. Three flasks of this medium were inoculated with *V. Dahliae*, and a similar number with a hyaline variant of *V. Dahliae*, *V. albo-atrum*, a

Text-fig. 3. Penetration of young sainfoin root through the wounds caused by the emergence of a lateral rootlet (× 100).

hyaline variant of *V. albo-atrum* and *V. nigrescens*, respectively.

The three species and both hyaline variants grew rapidly and covered the grains within 2–3 weeks. They differed, however, in the maximum viability of spores, mycelium and resting bodies (microsclerotia, resting mycelium and chlamydospores) as follows: *V. Dahliae* 7–8 weeks, *V. albo-atrum* 12 weeks; both hyaline variants 6 months; *V. nigrescens* 12 weeks.

From the results it is seen that rapid autostaling of all the *Verticillium* spp. takes place on this medium and that the toxic substances are produced most abundantly in the presence of black carbonized resting bodies.

(3) *Viability in potting soil*

Pots of soil of both types were inoculated with wheat-grain cultures and one pot of each was treated as follows: (*a*) waterlogged (approx. 100 % saturation) by standing in a tray of water, (*b*) watered every other day (approx. 50 % average saturation), and (*c*) watered once each week (approx. 20–30 % average saturation). Isolation attempts from every pot were made daily for 10 days after inoculation and then weekly for 25 weeks. The results throughout from partially sterilized and unsterilized soil were identical, and no differences were shown between the various pot treatments until after 15 weeks.

(i) *Days* 1–5. Plates from each treatment were crowded with *Verticillium* colonies visible to the naked eye 3–4 days after plating.

(ii) *Days* 6–10. A few colonies appeared 3–4 days after plating followed by a further crop of colonies about 4 days later.

(iii) *Up to 15th week.* Similar results to (ii), but number of isolates gradually decreasing.

(iv) *After 15 weeks. Verticillium* could not be isolated from the water-logged soils and after 22 weeks every attempt at isolation failed for all treatments.

In interpreting the above results it is appreciated that failure to isolate *Verticillium* by the method employed does not necessarily imply that viable fungus was entirely absent from the soils. The above data however do, it is suggested, enable a quantitative comparison to be made of the viable fungus present in the soil under the varied conditions of the experiment. On this assumption it is concluded therefore that *V. Dahliae* persisted equally well in partially sterilized and unsterilized soils and that the persistence of viability in the waterlogged soils was slightly less than that in the drained soils. The results also indicate that *V. Dahliae* persisted in the soil in a form yielding visible colonies within 3–4 days of transfer to plates and another form yielding colonies later, i.e. 7–8 days after transfer. As colonies of *V. Dahliae* from hyaline mycelium and conidia normally appear 3–4 days after plate inoculation, it is probable that these 'delayed' colonies originated from microsclerotia and that it was preponderantly, and later entirely, in this form that the fungus persisted in the soil from about the tenth day.

(4) *Comparative viability of* V. Dahliae *and its hyaline variant in potting soil*

If hyaline variants of *V. Dahliae* occur in nature as frequently as in artificial culture, and if (as the results of the previous experiment suggest) this fungus normally persists in the soil as microsclerotia, these variants might be expected to become extinct soon after they arise. As hyaline variants have been proved to be pathogenic by direct inoculation and re-isolation tests, such non-persistence might therefore account for the failure to isolate hyaline variants from naturally infected plant material. The following experiment was made to test this point.

During the first week of the months of January–June inclusive five pots of soil were inoculated with wheat-grain cultures of *V. Dahliae* and five with wheat-grain cultures of the variant, with two pots of uninoculated soil as controls. In the first week of June attempts were made to isolate the fungi from the inoculated soils, with the results shown in Table 3.

TABLE 3. *Isolation of* V. Dahliae *and variant from inoculated soil*

Month of inoculation	Isolations made in early June	
	No. of colonies of *V. Dahliae*	No. of colonies of variant
Jan.	Few (1)*	Nil (3)
Feb.	Few (1)	Nil (3)
Mar.	Fair number (1)	Nil (3)
Apr.	Many (1)	Nil (3)
May	Many (1)	Very few (2)
June	Many (1)	Many (1)

* Figures in brackets indicate number of isolation attempts.

Immediately after the last soil inoculation (i.e. during early June) a sainfoin seedling (Hunter) was transplanted into each plot with the results shown in Table 4.

TABLE 4. *Wilt of sainfoin in inoculated soil*

Month of inoculation	*V. Dahliae* No. of plants wilted out of 5 in inoculated soil	Variant No. of plants wilted out of 5 in inoculated soil
Jan.	5	1
Feb.	5	0
Mar.	5	0
Apr.	5	2
May	5	1
June	5	5

The six plants in control pots remained healthy.

The form of *Verticillium* (microsclerotial or hyaline) subsequently isolated from each plant in the inoculated series corresponded in every instance with the form with which the soil had been inoculated.

A heavy concentration of the microsclerotial form appears therefore to persist in the soil for at least 5 months, whereas, over the same period, the concentration of the variant quickly diminishes although it does not die out completely.

It may be concluded from these experiments that *V. Dahliae* hibernates in the soil mainly as microsclerotia and that, should a hyaline variant arise, its concentration will rapidly decline.

Environmental factors in relation to wilt incidence

Pots of uniform size (cast 32) and the Hunter strain of common sainfoin were used throughout the following experiments. Soil inoculations were made with wheat-grain cultures of *V. Dahliae* 4–5 weeks old. At the conclusion of the experiments all the plants were examined; without exception the wood of every wilted plant was found to be discoloured and *V. Dahliae* was invariably isolated from it. The wood of healthy-looking plants was always found to be normal in appearance and, as early attempts at isolation of the pathogen from samples of such plants were unsuccessful, it was therefore assumed that these were uninfected.

(1) *Soil moisture*

Workers on *Verticillium* wilts of different crops have arrived at varying conclusions on the effect of soil moisture on the diseases. Thus Pethybridge (1916), Van der Meer (1925) and Nelson (1937) concluded that drought is distinctly favourable to the disease, whilst Bewley (1922), Dowson (1923) and Harris (1936) believed that excessive moisture increased the intensity of attack. Rudolph (1931) stated that in California severe disease was observed in peach, apricot and prune orchards in which the soil varied from very dry to very wet.

The present investigation was conducted to determine whether variations in the soil water content affected the incidence of disease in sainfoin. Young sainfoin plants were grown in artificially contaminated farm soil taken from the vicinity of the roots of wilted plants at site 1, and in artificially contaminated potting soil containing different amounts of water.

The two soils were air-dried and their water-holding capacities determined—100 g. of each soil held approximately 40–45 g. of water when saturated. Fifty pots, each weighing 1000 g., were each filled with air-dried potting soil and fifty each with farm soil. Ten pots of each type of soil were brought to the following water-saturation points—30, 40, 50, 60 and 70 %.

V. Dahliae was then added to eight pots in each series, two additional pots being reserved as controls, and one sainfoin seedling was planted in each pot. Throughout the experiment, which continued for 7 months, the pots were kept up to their original weights, loss of water through evaporation and transpiration being thus made good. To minimize this loss the plants were kept in the humid atmosphere of a cool house. The obvious inaccuracies due to the increasing weight of the plants through growth were reduced by conducting the experiment during the months when growth was at a minimum. The results are summarized in Table 5.

The twenty control plants remained healthy, the best growth occurring in soil with 50 % water content; the vigour of the plants deteriorated with an increase or decrease in the water content. Growth on both soils was very similar.

It is concluded from the above results that an increase in the water content of the soil decreases the incidence of disease and that of the two soils ordinary potting soil is more favourable to infection.

(2) *Lime*

The effects of liming upon sainfoin in its relations to *V. Dahliae* were investigated because this crop normally grows most vigorously on calcareous soils. Bewley (1922), working with tomatoes, suggested that liming of the soil tended to reduce *Verticillium* infection, while Haenseler (1928), with eggplant,

Table 5. *Effect of soil moisture upon the* Verticillium *disease of sainfoin*

Water content %	No. of plants wilted out of 8 in each inoculated soil	
	Ordinary potting soil	Farm soi
30	8	5
40	6	5
50	7	2
60	5	3
70	2	0

Table 6. *Effect of lime upon* Verticillium *disease of sainfoin*

Amount of lime g. per pot	Initial *p*H of soil	Final *p*H of soil	No. of plants wilted out of 9 in inoculated soil
28·0	11·28	8·64	8
21·0	9·83	8·64	7
14·0	8·18	8·50	8
0	7·04	7·04	8

28·0 g. per pot = approx. 6 ton/acre.
The four control plants remained healthy.

maintained that increased alkalinity of the soil brought about by liming was favourable to *Verticillium* disease.

In the following experiment hydrate of lime was used with ordinary potting soil inoculated with *V. Dahliae*. Comparison was made between four levels of lime—nil (control), 14·0, 21·0 and 28·0 g. per pot in ten-pot replicates.

After the soils had been watered for 1 week the *p*H of each was determined and the fungus culture added to nine pots in each series, the remaining pot being kept as a control. A young sainfoin plant which had been growing in heavily limed soil for some time was transferred to each pot. At the end of the experiment, 3 months later, the *p*H of the soil was again taken. The results are summarized in Table 6.

These results show that sainfoin is still susceptible to *Verticillium* attack in intensely limed soil. The fact that the fungus remains active in very alkaline soils agrees with results obtained in cultural work where good growth of *V. Dahliae* was recorded on media at *p*H 9·6.

Most of this investigation was carried out during the tenure of a scholarship awarded by the Ministry of Agriculture and Fisheries and the Agricultural Research Council. The author wishes to express his thanks to Prof. F. T. Brooks, who suggested the investigation, for his interest and help; to the Cambridge Plant Breeding Institute for facilities and to the East Malling Research Station and the Agricultural Research Council for means to continue the work in conjunction with research on *Verticillium* wilt of hops under a special research grant. He is also indebted to Dr R. V. Harris for advice and criticism in the preparation of this paper.

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EXPLANATION OF PLATE 3

Fig. 1. Naturally infected sainfoin plant among healthy plants.

Fig. 2. Diseased sainfoin plant cut down the middle to show the discoloured wood of stems and roots.

Fig. 3. Sainfoin seedlings growing on Dox's agar in contact with *V. Dahliae*.

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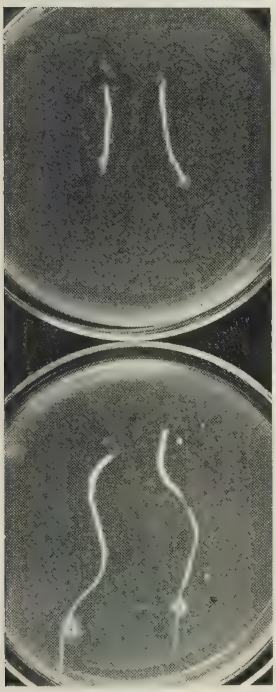

Fig. 3

Fig. 2

Fig. 1

Eyespot of wheat and barley in Scotland in 1944

By Mary D. Glynne, *Rothamsted Experimental Station, Harpenden, Herts.*

In August 1944, eyespot (*Cercosporella herpotrichoides* Fron.) was found in ninety out of 121 autumn-sown wheat crops distributed over twelve Scottish counties. It was abundant enough in forty fields to be likely to harm subsequent crops, and was causing obvious loss in eleven crops. The disease was found in seventeen out of eighteen spring-sown barley crops, more than half the straws being infected in seven of them. About 4% of the wheat inspected was lodged mostly by eyespot and about 38% of the barley mostly non-parasitically. Eyespot increased with the frequency of wheat and barley in the two preceding years, but a few infected crops occurred on fields where no wheat or barley had been grown for many years. In Scotland, where the atmosphere is more humid, eyespot tends to increase more rapidly than in similar rotations in England; lesions are found higher up the straw and the disease is much more prevalent on spring-sown barley. The long rotations practised in Scotland prevent more extensive damage by eyespot.

Introduction

In 1941, when surveys of wheat crops in the southern half of Britain were reported by Glynne (1942), there were no records of eyespot of wheat (*Cercosporella herpotrichoides* Fron.) occurring farther north than Nottinghamshire and Denbighshire. Later, unpublished reports showed that it occurred in northern England and in Scotland. Dennis (1944) stated that it was known to be widespread in the Lothians, Fife and Morayshire, where it was thought to be responsible for more whiteheads than take-all. He considered that extensive lodging due to eyespot was uncommon, but recorded that instances of 35 and 50% loss had been reported in recent years with the increase in cereal cropping.

A survey of winter-sown wheat and of a few spring-sown barley crops was carried out between 7 and 19 Aug. 1944 in twelve of the chief wheat growing counties in Scotland.

Methods

There was a slight intentional bias in favour of land where wheat or barley had been grown recently. In each crop, eye estimations were made of the percentage area lodged, the percentage straws straggled and those showing whiteheads. Affected straws were examined and the causes recorded; the selective examination of straggled straws proved to be the most rapid method of discovering the presence of eyespot. Counts were made of 20–50 straws scattered about the field, in the upright and lodged parts of the crop separately, recording the numbers of healthy straws and those with slight and severe eyespot lesions, take-all and sharp eyespot. The method sufficed for classification of crops into five grades of eyespot infection, 0, 1–20, 21–50, 51–70 and 71–100% straws infected, slight and severe lesions being included.

Incidence and causes of disease

(*a*) *Lodging.* When lodging is caused by eyespot, the diseased straws bend at an acute angle in the middle of the lesion and lie close to the ground, but when it is non-parasitic, the straws curve more gradually down to soil-level. Lodged areas, varying from 5 to 90% of the crop, were recorded in twelve wheat fields as due to eyespot and 10 and 50% of two other crops were lodged non-parasitically, so that more than three-quarters of the lodging seen in wheat was due to eyespot. At the time of the survey the mean area estimated as lodged in the 121 wheat crops examined was about 4%, there being most in Kincardine and least in Dumfriesshire, but Dr Gray reported that subsequent heavy rain before harvest greatly increased the area lodged in the northern area.

Of the barley inspected 38% was estimated as lodged, mostly non-parasitically, but when eyespot was severe it seemed to have increased lodging and made the crop lie flatter on the ground.

(*b*) *Straggling in wheat.* Straggling, i.e. the falling over of individual straws among upright ones, occurred in most crops in which eyespot was found, generally affecting less than 1% of the straws, but up to 5% were noted in thirteen and 5 to 50% in seven other crops. Straggling was generally caused by eyespot and occasionally by sharp eyespot.

(*c*) *Whiteheads in wheat.* White early-ripened heads were found associated with eyespot lesions at the straw bases in sixty-one crops, with take-all in forty-six, with sharp eyespot in six, with a translucent water-soaked appearance of the straw and leaf sheaths (possibly frost or insect damage) in four, and with no obvious cause in four crops. Two or more agents were often found causing whiteheads of different plants in the same crop. Take-all, though causing whiteheads in forty-six crops, was often responsible for only a small proportion in a crop, while the majority of whiteheads were due to eyespot. In Dumfriesshire and Aberdeenshire, take-all accounted for more whiteheads than did eyespot, but in most counties Dennis's observation that eyespot was responsible for more whiteheads than take-all was confirmed.

Incidence of eyespot on wheat

(*a*) *Regional.* Eyespot was found in ninety out of the 121 autumn-sown wheat crops inspected, distributed between the twelve Scottish counties visited. The highest proportion of crops with more than half their straws infected was in Kincardine; the lowest was in Dumfriesshire and Aberdeenshire, where wheat occurs less often in the rotation than in most other counties.

Table 1 shows that a third of the crops inspected had more than 20% of the straws infected. This would probably be enough to produce severe infection in subsequent susceptible crops, and precautions should be taken to avoid trouble especially in the two following seasons. In very wet seasons or districts, crops with less than 20% infected straws might also carry enough infection to harm subsequent wheat or barley crops.

Only a very general idea of the loss involved can be obtained from this type of survey. Although twenty-eight crops had more than 50% of their straws infected, obvious loss was seen in only eleven, being severe in five of them. Later lodging probably added to the loss obvious at the time of survey. Three heavily infected crops yielded only 11–13 cwt./acre, though the preceding, presumably healthy, wheat crops gave 31–33 cwt./acre and there was little doubt that much of the reduction in yield was due to eyespot.

(*b*) *Effect of rotation.* The tendency for eyespot to increase with the frequency of wheat and barley in the rotation, has been noted in every country in which the disease has been studied. But very few data have been published whereby the degree to which rotation controls infection by eyespot can be assessed, or whereby the intensity of infection in similar rotations in different seasons and regions can be compared. Further, there are conflicting reports without supporting details on the effect of preceding crops such as peas or beans. Buddin (unpublished report) has pointed out that such crops often include volunteer plants which may carry infection; information on previous cropping is thus essential in the interpretation of these reports. Detailed records are needed from many localities from which particular types of rotation can be selected for study. Table 2 shows that the frequency with which wheat or barley have been grown in the previous years tends to increase the incidence of eyespot, but is no certain guide. Thus, the second successive wheat crop, though often heavily infected, sometimes escaped infection altogether; and severe infection, though usually preceded by wheat or barley in one of the two previous years, occasionally occurred on land where no wheat or barley had been grown for many years. Factors other than preceding wheat or barley crops evidently play an important part in determining the incidence of eyespot.

TABLE 1. *Incidence of eyespot in Scotland. Counties arranged from south to north*

	No. of fields						
	% straws infected with eyespot						
County	0	1–20	21–50	51–70	71–100	Total	Mean % infection
Dumfries	10	1	0	1	0	12	6
Roxburgh	1	3	0	0	0	4	26
Berwick	2	0	0	2	1	5	
East Lothian	0	7	3	1	1	12	27
Mid Lothian	1	3	1	1	1	7	30
Fife	3	8	0	0	3	14	24
Perth	0	1	0	0	1	2	—
Angus	0	6	0	2	2	10	35
Kincardine	1	4	2	0	7	14	50
Aberdeen	7	4	0	1	0	12	8
Moray	3	5	1	0	1	10	17
Ross and Cromarty	3	8	5	1	2	19	26
Total in 12 counties	31	50	12	9	19	121	25

A summary of the degree of infection in relation to different types of cropping is shown in Table 3. The numbers of crops following different types of rotation are insufficient to provide proof, but indicate probable tendencies which are shown by the number of crops in each infection group and the estimated mean percentage infection.

Grass seems less favourable to eyespot than arable without wheat or barley (Table 3, cf. lines 1 and 2 with 3 and 5 with 6). Wheat or barley 3 or 4 years earlier (line 4) followed by arable did not appear to increase eyespot in Scotland, though it seemed to do so in England in 1941, suggesting that the fungus may survive longer in the absence of the known susceptible crops in England than in Scotland. Wheat or barley in either of the two previous years, 1942 or 1943, greatly increased infection, and this was further increased by wheat or barley in 1940 or 1941

(cp. lines 6 and 7 with 8). Except for the rotation shown in line 4, similar rotations in Scotland showed a similar effect on eyespot to that already found in southern Britain in 1941, but infection was generally more severe in Scotland than it had been in southern Britain. This effect is also shown by the fact that England had more than 20 % of their straws infected. Comparison with annual surveys carried out at the Rothamsted Experimental Station during the past 7 years showed that the tendency to a greater infection in similar rotations in Scotland was not merely a seasonal effect. Eyespot infection of about

TABLE 2. *Previous crops in relation to eyespot*

Arranged according to occurrence of wheat and barley crops

Percentage straws infected by eyespot in 1944 wheat crop

0	1–20	21–50	51–70	71–100
4 G G G G	2 . . G G	G F P P	O H R O	G G G P
7 G G G O	2 G G G O	O G G G	W T O P	O T O H
3 G G O O	G G G P	O P T Be	. G G W	W H P O
G O O O	G G O P	B H H P	G G O W	O P W P
2 G G G T	. G O T	G O B R	O T O B	O T B P
G G G P	G O O P	G G G W	B H P W	B P W P
G G G Be	G F P P	2 G G P W	G R W W	W B W Rp
G G G F	O H G P	O P T B	. W W W	. G G W
O G O {P, Be}	O H S P	W O F W	W W W W	G O T B
.	O H O P	W T P W		G P P W
T O G O	3 O T O P	G {W, B} T B		P P P W
2 P W G G	O O T O			P O T W
P W Be M	R O H O			T O P W
T B H O	. . O P			H O P W
B B H P	3 W G G G			W P P W
G O T {O, B, Pe}	2 B G G O			B G G W
	B R G G			G R W B
	2 B G O P			B P W W
3 G G G W	W O H P			W W W W
	. B H P			
	2 T B G G			
	G W O P			
	P W O H			
	W W H P			
	G G W {O, Pe}			
	G O W O			
	P P W S			
	O P W P			
	O T B S			
	. T B P			
	2 G G G W			
	3 G G O W			
	G O P W			
	2 T O P W			
	P O P W			
	O Rp R W			
	W O G W			

Figures denote the number of crops following the same rotation when this was more than one.

W, wheat; G, grass; P, potatoes; M, mangolds; Be, beans; B, Barley; H, hay; T, turnips; Rp, rape; F, fallow; O, oats; R, roots; S, sugar beet; Pe, peas.

Thus 2BGOP means that two of the 1944 wheat crops had been preceded by barley in 1940, grass in 1941, oats in 1942 and potatoes in 1943.

among crops which had not been preceded by wheat or barley during the previous 4 years, 13 % in Scotland and none in southern Britain, had more than 20 % straws infected. Among crops which had been preceded by two or more wheat or barley crops in the previous 4 years, 88 % in Scotland and 77 % in 20 % of the straws was generally found in the third wheat crop after grass at Rothamsted; whereas, in Scotland, more than 20 % of the straws were infected in six out of fourteen fields in which the second wheat crop was taken after grass (with not more than one arable crop intervening).

Eyespot, estimated as affecting 50, 70, 35, 75, 70, 90 and 50% of the straws, was found in seven crops where no wheat or barley had been grown for 4, 4, 6, 6, 7, 7 and 14 years respectively. Such exceptions emphasize the need for studying the factors other than the effect of known susceptible crops, on the incidence of eyespot. In Scotland, eyespot lesions were found much higher up the straws than in England, where they are generally within 2–3 in. of ground-level. It is suggested that infected straw, cut below the level of infection and applied to previous crops in dung, may prove to be a source of infection in damper regions, though it is not regarded as a potential danger farther south where lesions generally occur below the level at which the straw is cut. Weeds may also prove to be a means of carrying infection.

TABLE 3. *Incidence of eyespot in relation to previous crops*

(1) Line*	(2) Previous crops 1940	1941	1942	1943	(3) No. of wheat crops in 1944 % straws with eyespot 0	1–20	21–50	51–70	71–100	Total	(4) Mean % eyespot approx. in Scotland in 1944		(5) Survey 1. 1941 wheat preceded by similar crops No. crops	Mean % eyespot approx.
1	G	G	G	G	4	2	0	0	0	6	3		11	3
2	G	G	G or A	A	15	3	0	0	1	19	6	11 (+1)	19	1
3	G or A	A	A	A	3	13	3	1	1	21	18 (+1)		14	6
4	1 or 2 W or B, 0–1 G or A		A	A	5	15	1	1	1	23	14		9	30
5	G	G	1 W or B, 1 G or A		3	6	3	2	1	15	25		1	—
6	G or A	A	W or B	A	0	5	1	0	2	8	32 (+1)	41 (+1)	36	40
7	G or A	A	A	W or B	1	5	1	1	6	14	47 (−1)			
8	1 W or B, 1 G or A		1 W or B, 1 A		0	1	3	1	3	8	54 (+2)	63 (−2)	22	49
9	2–4 W or B, 0–2 G or A				0	0	0	3	4	7	74 (−6)			

A, arable, including 1 year leys, with no wheat or barley. G, permanent grass, or at least 2 years ley. W, wheat. B, barley.

The mean percentage eyespot infection in Scotland, calculated from the mean values in each infection group, is shown in column (4). Corresponding figures were obtained from survey 1 which was carried out in southern Britain in 1941 (Glynne, 1942) in the same way as the Scottish survey, except that the two infection groups 21–50 and 51–70 were included in one with 21–70% straws infected. These are shown in column (5). The slight adjustment necessary in some instances to make the figures obtained in Scotland strictly comparable to those in England is shown in brackets in column (4).

* Lines are numbered solely for convenience in referring to previous cropping on p. 36.

Eyespot of barley

Eyespot was found in seventeen out of eighteen spring-sown barley crops; four, six, three and four crops had respectively 1–20, 21–50, 51–70 and 71–100% straws infected. Neither straggling nor whiteheads were seen; the disease, though less severe than on autumn-sown wheat, seemed to have caused damage in seven crops by increasing the tendency to lodge and making the crops lie flatter on the ground. It had previously been supposed that eyespot attacked spring-sown crops too late to cause harm to them, though they carried infection to subsequent crops. The occurrence of eyespot more commonly and more severely on spring-sown barley in Scotland than in southern England is therefore noteworthy.

Take-all, Ophiobolus graminis (*Sacc.*) *Sacc.*

Take-all was found in fifty-two of the wheat crops; forty-three had less than 1%, eight had 1–5% and one had 10% straws infected. The disease causes greater loss for a similar percentage of obviously infected straws than does eyespot. It was often found near the gate or at the edge of crops in which eyespot predominated in the main part. In two fields in which wheat followed wheat, take-all appeared to have caused more damage than eyespot; it was more common than eyespot in Dumfriesshire and Aberdeenshire, but in most counties much more eyespot than take-all was found.

Sharp eyespot, Corticium (Rhizoctonia) Solani (*Prill. & Delacr., Bourd & Galz.*)

This was recorded in thirty-four crops in nine counties. It was most common in Aberdeenshire where it was found in more than half the crops inspected. Generally less than 1 % of the straws were infected, but 1–5 % infected straws were found in three crops, and 6–20 % in one crop in which patches with more than 20 % infected straws occurred with disease free areas in between. Sharp eyespot often ascended the straw to a height of 12 in. above soil level; it was often superficial, but sometimes penetrated deeply, causing straggling of 1–5 % of the straws in two crops, and much less often causing whiteheads. It was more common than previously noted in England, but did not seem to be responsible for any appreciable loss of crop, though it is possible that it may kill plants early in the season.

DISCUSSION

Although much more wheat and barley have recently been grown in Scotland than in pre-war years, the rotations generally practised are variations of the six-course in which grass alternates with roots, cereals and leguminous crops. They thus tend to include wheat and barley less often than do the rotations generally practised in southern England, and so tend to check the development of eyespot. The rainfall Apr.–July inclusive 1944 varied from 8·9 to 11·2 in. in eight Scottish counties for which figures were obtained, while that at Rothamsted was only 5·6 in. The humid conditions which consequently prevailed for a greater part of the spring and summer in the north than in the south, must have favoured the disease. The tendency for eyespot to develop more rapidly under similar rotations, to ascend higher up the straw, and to attack spring-sown barley more severely in Scotland than in southern England, support the view that climatic conditions in Scotland were more favourable to eyespot than those found in southern England. The rapid development of eyespot with close cereal cropping may be one of the several factors which have prevented the increase of wheat growing in the more humid regions.

It is a pleasure to record my thanks to Dr Foister for arranging this survey, to Dr Dennis and Dr Elizabeth Gray for unstinted help in carrying it out, to the College, County and W.A.E.C. officers for placing their intimate knowledge of local conditions at my disposal, and to many farmers for their courtesy and patience.

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The control of narcissus leaf diseases

III. *Sclerotinia polyblastis* Greg. on *Narcissus tazetta* var. Soleil d'Or

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In the extreme south-west of England *Narcissus tazetta* grown for the outdoor flower crop is regularly attacked by narcissus fire (*Sclerotinia polyblastis*). A randomized strip experiment on the variety Soleil d'Or laid down in 1937, in which half the plots were sprayed each season, gave data on the effect of controlling this disease on the number and quality of flowers produced in 1939, 1940 and 1941, and on the weight and grade of bulbs lifted in 1941.

There was an average increase of 26 % in the number of flowers produced on sprayed plots, and a 35% increase in weight of bulbs. There was no evidence of a cumulative improvement because most of the gain in one year appears to have been immediately expended in increased flowers in the following season, but the sprayed plots maintained a higher general level throughout. The quality was improved by spraying, mainly by the addition to the inflorescence of an average of one extra 'bell'. Although not cumulative, there was a residual effect shown by the increased yield of bulbs in 1941 after a season in which only one post-flowering spray had been applied.

The effect of treatment on the date of flowering (anthesis) was negligible and its direction depended on the season. In this respect Soleil d'Or differs from Golden Spur, in which spraying induced the marked retardation of flowering noted in 1938 and 1939.

Introduction

The experiments described here record the effect on yield of preventing narcissus fire, due to *Sclerotinia* (*Botrytis*) *polyblastis*, which is the chief fungus disease of the *Narcissus tazetta* varieties grown for commercial flower production in the open in the south-west of England (Beaumont, 1930; Gregory, 1937, 1939).

When the work started there was no information on methods of controlling narcissus fire, or upon the effects of the disease, if any, on the yield of bulbs and flowers. Earlier papers (Gregory, 1940*a*, *b*) showed that when white mould (*Ramularia vallisumbrosae*) on *Narcissus pseudo-narcissus* was controlled by copper sprays, increased yields of flowers and bulbs were obtained in the following season, but that there was a marked retardation of flowering date.

It was obviously impossible to measure the loss of crop due to *Sclerotinia polyblastis* until some method of controlling the disease could be found to enable a comparison to be made between diseased and healthy plants growing under the same conditions. For this purpose it matters very little whether the method of control adopted is economically practicable or not.

Treatments

Preliminary tests were started in 1937 on a commercial field of Soleil d'Or which had been planted in 1934 at the Isles of Scilly Experiment Station, St Mary's, and observations were continued on the same plots until the end of 1939. Single plots each consisting of several beds were treated as summarized in Table 1.

Meanwhile it had been decided to test the effect of spraying this variety with Bordeaux mixture regularly over a period of years. The experiment was laid down in the summer of 1937 and consisted of ten pairs of small beds of narcissus Soleil d'Or. The foliage on one bed of each pair, chosen at random, was sprayed with Bordeaux mixture once or twice each season from 1938 to 1941. The number of flowers produced from each bed was recorded in 1939, 1940 and 1941. In the summer of 1941 the bulbs were lifted and weighed and records were then discontinued on account of the war.

The site chosen was typical of Scillonian flower farms. The field measured about 50 × 30 yd. and was surrounded on all sides by windbreaks of veronica hedges with occasional elm trees: consequently, the end plots were liable to severe competition from the surrounding trees and hedges. The bulbs were planted after early potatoes which had been manured with wool waste.

The stock of bulbs used was lifted from an adjacent field and stored for some weeks in an unheated glasshouse. Small non-flowering 'rounds', weighing approximately 45 lb. per 1000, were selected for planting in the plots, other grades being used elsewhere. The plots were planted on 1 Sept. 1937 by setting in plough furrows. They were arranged in beds 4 ft. 6 in. wide by twenty furrows

* These studies were carried out while at Seale-Hayne Agricultural College, Newton Abbot, Devon.

long. Each bed contained 340 bulbs, but the number was subsequently reduced by an unusually heavy attack by bulb fly, mainly during the first season, and the number of plants surviving in each bed in 1939 is shown in Table 10.

The fungicide used throughout the experiment was Bordeaux mixture containing 4 lb. copper sulphate, 4 lb. fresh hydrated lime and 40 gal. water. A wetting agent was always added (usually Agral 2 at 6 oz./40 gal.). Spray was applied with a pneumatic knapsack machine at the rate of about 120 gal./acre. Each year one or two applications were given, one soon after the flowers had been picked, and a second if possible about 1 month later.

In 1938 spraying was carried out on 24 Feb. and 28 Mar. As commonly happens with newly planted bulbs very little disease appeared on the foliage of the plots, and on 4 May it was recorded that no spray damage was evident on the treated foliage and that both treated and control plots appeared to be ripening off fairly equally. The yield of flowers in 1939 showed that the sprayed plots had, however, substantially benefited.

After the usual cultivations during the previous summer the plots were hand-weeded in Jan. 1939 after the flower buds had appeared above ground. The plots were sprayed on 15 Feb. and again on 15 Mar. By 28 Apr. there were obvious differences to the eye between sprayed and unsprayed beds, but the foliage of all plots was beginning to go down, especially on unsprayed plots. By 18 May the control plots were estimated by eye to have lost about half their leaf area as a result of attack by *Sclerotinia polyblastis*, while the sprayed plots had only small lesions and, with their bigger leaves, could be recognized at a distance. On 10 June the control plots were dead, while the sprayed plots were still partly green.

Jan. 1940 was exceptionally cold in the Isles of Scilly, and plots 1–3, which were situated at the bottom of the slope above a hedge, suffered particularly from frost and wind damage. The plots were sprayed on 17 Feb. and 17 Apr., and the prolongation of life of the protected leaves appeared to be similar to the previous year.

In 1941 the plots were sprayed only once after flowering, on 7 Apr. Control of disease on the sprayed plots was as usual considered satisfactory.

In general it may be said that the stock was subject to the usual epidemic of narcissus fire in each of the years 1939–41, and that Bordeaux mixture gave good visual control and prolonged the life of the foliage by several weeks in each season. As a secondary effect, possibly attributable to the longer retention of foliage, there was an obvious reduction on sprayed plots of the quantity of *Oxalis cernua*, a troublesome weed in Scilly.

TABLE 1. *Preliminary test at St Mary's, Scilly*

Date	Cuprous oxide plot Beds 3–11	Unsprayed control Beds 12–18	Bordeaux mixture plot Beds 19–24
1937: 8 Dec.	Sprayed 0·125 % Cu as cuprous oxide		Sprayed ·4-4-40 Bordeaux with Agral 2
1938: 27 Jan.	Do.		No spray as flowers beginning to open
24 Feb.	Do.		Sprayed as 8 Dec.
4 May	Appreciably better than control	Foliage badly attacked *S. polyblastis*	Foliage green and standing well
1939: 14 Jan	Flower count: 120 per 100 plants	109 per 100 plants	144 per 100 plants
5 Feb.	No spray		Sprayed Bordeaux mixture as above
16 Mar.	Sprayed Cu_2O as above		Do.
20 July		Bulbs lifted	
12 Nov.		Produce of the following beds analysed	
		Beds 15–18	Beds 19–22
		Bulb wt.: 90 lb./1000	118 lb./1000
		Bulbs cut open and flower buds counted: 75 buds/100 bulbs	113 buds/100 bulbs

FLOWER-CROP RECORDS

Table 2 shows the number of 'flowers' harvested from the plots in each of the years 1939–41. The bulbs had been graded as non-flowering rounds when planted and produced only about half-a-dozen flowers in 1938. The 1939 figure is based on a count of the buds made before picking started. The 1940 and 1941 records are based on the totals picked from each plot on each picking occasion. (The term

'flower' in the *tazetta* group is applied in common language to what is botanically a cymose inflorescence. The true flower is known to growers as a 'bell' or 'pip'.)

A comprehensive analysis of variance showed the effects of treatments, blocks and years to be highly significant. The increase in crop on sprayed plots amounted to 20% in 1939, 18% in 1940 and 37% in 1941 over the unsprayed controls, or approximately 26% as an average over all seasons. The almost consistently better performance of plots towards the because drift of spray from treated to control plots should tend to increase the yield from untreated plots and, similarly, spore drift from control plots would in time contaminate the treated plots. Small, contiguous plots are ill-adapted to the study of epidemic foliage diseases because of the effect of one plot on its neighbours.

In 1940 and 1941 studies were made on the effect of spraying on anthesis. The retardation of sprayed narcissus Golden Spur has already been shown to be a serious factor in spraying crops of this variety

TABLE 2. *Total numbers of flowers picked from sprayed and unsprayed plots*

Plot	Unsprayed plots			Total	Plot	Sprayed plots			Total
no.	1939	1940	1941	1939–41	no.	1939	1940	1941	1939–41
1	118	152	151	421	2	121	173	193	487
3	95	163	184	442	4	123	217	272	612
6	112	195	215	522	5	151	232	280	663
7	122	189	201	512	8	154	266	325	745
10	138	250	267	655	9	188	259	331	778
11	171	243	287	701	12	161	264	258	683
13	168	272	288	728	14	164	270	374	808
15	144	258	276	678	16	185	316	403	904
18	148	226	230	604	17	158	293	368	819
19	88	204	204	496	20	163	251	256	670
Totals:	1304	2152	2303	5759		1568	2541	3060	7169

TABLE 3. *Anthesis on sprayed and unsprayed plots*, 1940

	Unsprayed plots			Sprayed plots		
Picking date	No. picked	Total picked up to date	Total %	No. picked	Total picked up to date	Total %
11 Jan.	75	75	3·5	94	94	3·7
26	210	285	13·3	190	284	11·2
27	51	336	15·6	35	319	12·5
29	235	571	26·6	262	581	22·9
31	134	705	23·8	153	734	28·9
2 Feb.	190	895	41·6	215	949	37·3
6	457	1352	62·9	556	1505	59·2
8	212	1564	72·8	310	1815	71·5
10	62	1626	75·6	57	1872	73·8
15	78	1704	79·4	96	1968	77·4
19	204	1908	88·8	275	2243	88·4
23	212	2120	98·5	250	2493	98·2
4 Mar.	32	2152	100·0	48	2541	100·0
Totals	2152			2541		

middle of the field is worth notice and suggests other problems needing investigation, because the crop appears to be reduced to one-half by some such factors as proximity of hedges. As already noted, plots 1–3 suffered from exposure in 1940. Plot 19 was also abnormal because, by 1941, it had developed a bare patch of about 1 sq. yd. where the bulbs had been killed by *Rosellinia necatrix*. (These white root-rot patches commonly develop in Scilly where piles of vegetable matter are left to rot on the surface.)

The differences in yield would probably have been still larger if whole fields could have been sprayed, grown for early flowers. It was therefore important to look for a comparable effect on anthesis in Soleil d'Or. In 1940 and 1941 the number of flowers picked from each bed on each picking date was recorded and the results are shown in Tables 3 and 4. Picking was carried out in both seasons under commercial conditions in which neither the interval between pickings nor the stage in which the flower was picked were standardized. The interval between successive pickings depended on the weather, and the beds were picked over when enough flowers had opened to make gathering worth while. In general,

the inflorescence was picked when one of its flowers was open, but with windy weather threatening, the picking might be 'closer' to avoid loss of flowers by mechanical damage. However, on each occasion standards were similar for both sprayed and unsprayed plots, and the records cover the entire season, so the lack of standardization cannot invalidate the conclusions drawn.

The records thus appear to indicate a slight effect of spraying on the date of flowering of the order of 1 day. However, the effect differed in its direction in different years. In 1940 the sprayed plots were slightly later in flowering, while in 1941 they were slightly earlier. This effect is quite different in magnitude from that noted with the variety Golden Spur, where there was a retardation due to spraying of from 4 to 8 days. It is unfortunate that similar studies with Golden Spur had to be discontinued before 1940, because we do not know whether the effect in that variety might not also be capable of seasonal reversal. It is likely, however, that the effect depends on the presence of living foliage in Golden Spur retarding the initiation of the following season's flower bud, which is normally laid down very early. *Narcissus tazetta* has apparently different physiological requirements, and flowering is favoured by warmth in summer. Studies carried out by one of us (G.W.G.) have shown that the flower bud is not formed until much later than is the case with daffodils (*N. pseudonarcissus*), and its initiation is therefore not likely to be delayed by prolonging the life of the foliage. As judged by the seasons 1940 and 1941 the effect of spraying on anthesis is negligible in Soleil d'Or.

TABLE 4. *Anthesis on sprayed and unsprayed plots*, 1941

	Unsprayed plots			Sprayed plots		
Picking date	No. picked	Total picked up to date	Total %	No. picked	Total picked up to date	Total %
3 Feb.	121	121	5·2	213	213	6·7
8	190	311	13·5	284	497	15·7
10	298	609	26·4	462	959	30·3
12	426	1035	44·6	563	1522	48·2
15	563	1598	69·4	792	2314	73·2
16	226	1824	79·1	275	2589	81·9
18	253	2077	90·2	307	2896	91·6
21	124	2201	95·5	144	3040	96·1
7 Mar.	70	2271	98·4	85	3125	98·9
9	32	2303	100·0	35	3160	100·0
Totals	2303			3160		

TABLE 5. *Bell count on* 31 *Jan. and* 2 *Feb.* 1940, *and* 16 *Feb.* 1941 (*mean number of flowers per inflorescence*)

	Unsprayed plots				Sprayed plots		
	1940		1941		1940		1941
Plot no.	31 Jan.	2 Feb.	16 Feb.	Plot no.	31 Jan.	2 Feb.	16 Feb.
1	6·9	7·4	6·9	2	7·5	7·1	7·0
3	8·5	8·1	7·1	4	10·5	9·8	7·8
6	8·4	9·1	7·3	5	9·9	9·4	7·8
7	8·1	7·9	8·1	8	9·3	9·1	8·7
10	10·8	9·6	8·0	9	10·3	8·6	8·4
11	8·6	8·3	7·3	12	8·5	8·4	8·3
13	8·6	8·2	7·9	14	10·9	9·2	8·3
15	9·6	9·3	8·3	16	11·8	9·1	8·6
18	7·1	7·4	7·6	17	12·7	10·5	8·8
19	7·8	8·3	7·1	20	8·9	9·4	7·5
Mean	8·44	8·36	7·56		10·03	9·06	8·12

FLOWER QUALITY

Previous experiments with narcissus Golden Spur showed that sprayed plots produced heavier flowers and stems than unsprayed plots. The stems were not of any greater length, but their extra weight was due to increased thickness. In assessing quality in a *N. tazetta* variety like Soleil d'Or an extra criterion is available, that of the number of flowers or 'bells' in the inflorescence. Accordingly, in 1940, on two dates in mid-season, all flowers picked from the plots were examined for bell count, stem length, weight of inflorescence and pedicel cut at base of spathe, and

TABLE 6. *Mean wt. (g.) of inflorescences on sampling dates*

Unsprayed plots				Sprayed plots			
	1940		1941		1940		1941
Plot no.	31 Jan.	2 Feb.	16 Feb.	Plot no.	31 Jan.	2 Feb.	16 Feb.
1	2·50	2·57	2·33	2	2·70	2·42	2·40
3	2·19	2·72	2·46	4	3·08	3·05	2·80
6	2·75	2·89	2·85	5	3·34	3·05	2·69
7	2·75	2·61	3·07	8	3·05	2·97	3·15
10	2·89	3·00	2·89	9	3·31	2·69	2·90
11	2·87	2·77	2·66	12	2·85	2·75	3·09
13	2·89	2·60	2·80	14	3·29	2·85	2·76
15	3·10	2·85	3·09	16	3·60	2·78	3·06
18	2·22	2·37	2·76	17	3·00	3·06	3·03
19	2·55	2·56	2·50	20	2·77	2·68	2·71
Means	2·67	2·69	2·74		3·10	2·83	2·85

TABLE 7. *Mean wt. (g.) of stems on sampling dates*

Unsprayed plots				Sprayed plots			
	1940		1941		1940		1941
Plot no.	31 Jan.	2 Feb.	16 Feb.	Plot no.	31 Jan.	2 Feb.	16 Feb.
1	7·00	7·22	6·07	2	8·00	7·06	6·90
3	7·57	7·73	6·54	4	8·62	8·36	8·27
6	7·16	8·12	7·66	5	8·68	8·54	8·43
7	7·74	7·61	8·17	8	7·86	8·31	9·12
10	7·56	8·57	7·28	9	8·58	7·48	8·56
11	7·44	7·15	7·53	12	7·40	8·00	8·53
13	7·11	7·40	7·29	14	8·30	7·85	8·54
15	7·50	7·53	8·33	16	9·20	7·56	8·85
18	6·28	6·62	7·13	17	8·00	9·06	8·76
19	6·34	7·71	6·67	20	6·92	8·36	8·10
Means	7·17	7·57	7·27		8·16	8·06	8·41

TABLE 8. *Mean stem length (mm.) on* 1940 *sampling dates*

Unsprayed plots			Sprayed plots		
Plot no.	31 Jan.	2 Feb.	Plot no.	31 Jan.	2 Feb.
1	266	257	2	303	266
3	259	257	4	284	247
6	262	247	5	264	262
7	254	241	8	264	245
10	258	250	9	264	244
11	248	231	12	253	254
13	242	239	14	245	244
15	234	218	16	256	266
18	256	250	17	245	236
19	255	268	20	288	291
Mean	253	246		266	253

weight of stem. In 1941 similar information was available for one picking date.

The mean number of 'bells' as judged by counts on all flowers picked on these three sampling occasions was consistently about 10% greater on sprayed plots. Spraying has clearly increased the weight of both flowers and stems. The increase in stem length, although consistent, is slight. The increased weight of the flower is apparently due to the addition, on the average, of about one extra 'bell' to the inflorescence.

BULB YIELD

In August 1941, 4 years after planting, the bulbs were lifted, weighed and graded, and the experiment was then discontinued. Table 9 shows the total weight lifted from each plot. The sprayed plot of each pair gave a higher yield than the unsprayed, and a test of significance showed values of t corresponding to odds of over 1 in 50. The total number of bulbs of all grades was not recorded. Table 10 gives the number of bulbs in the various flowering grades from 13 cm. upwards.

In each block the greater weight of bulbs was lifted from the sprayed plot, and the mean increase in total

TABLE 9. *Yield of bulbs (lb.) compared with weight planted*

	Unsprayed plots				Sprayed plots			
Plot no.	Wt. (lb.) 1937	No. 1939	Wt. (lb.) 1941	Plot no.	Wt. (lb.) 1937	No. 1939	Wt. (lb.) 1941	Block total (lb.) 1941
1	14	277	28	2	14¼	217	32	60
3	13½	237	31	4	14¼	257	50	81
6	15¼	268	39	5	15	252	50	89
7	15	257	38	8	14½	283	54	92
10	14½	301	44	9	15¾	285	55	99
11	15	312	50	12	15½	326	61	111
13	16¾	328	54	14	15¾	324	70	124
15	15½	324	67	16	15½	310	84	151
18	16¾	291	53	17	15½	301	83	136
19	15½	287	42	20	15¼	282	63	105
Total	151¾	2882	446		151¼	2837	602	1048

TABLE 10. *Numbers of bulbs of flowering size (from 13 cm. upwards)*

	Unsprayed plots				Sprayed plots				
Plot no.	13 cm.	14 cm.	15 cm. and up	Total 13 cm. and up	Plot no.	13 cm.	14 cm.	15 cm. and up	Total 13 cm. and up
1	63	41	9	113	2	61	62	26	149
3	50	54	25	129	4	42	75	89	206
6	46	74	56	176	5	30	61	126	217
7	41	71	65	177	8	21	64	139	224
10	31	85	97	213	9	22	62	152	236
11	43	86	90	219	12	27	57	175	259
13	52	75	130	257	14	21	63	180	264
15	44	84	90	218	16	9	66	152	227
18	34	86	98	218	17	18	48	130	196
19	38	75	71	184	20		(records incomplete)		
Total*	404	656	660	1720		251	558	1169	1978

* Omitting plots 19 and 20.

bulb weight was about 34%. The general effect of spraying on the grades of bulbs lifted was to increase the total number of bulbs of flowering size by about 15% and to increase the 15 cm. grade by about 77% at the expense of the 13 and 14 cm. grades. There was, therefore, every prospect that had the bulbs been replanted and crop records continued as originally planned before the war, the sprayed plots would have given an increased flower crop of better quality again in 1942.

The results obtained should not necessarily be interpreted as evidence that spraying with Bordeaux mixture is a commercially feasible control measure. Spraying of any kind is highly inconvenient in the climate and working conditions of the south-west, but, in view of the obviously large effect of the disease on the flower and bulb crop, search should obviously be made for simpler control measures based on good cultural practices. The significance of this experiment must be regarded merely as a stage in the investigation of this and other narcissus diseases. Meanwhile, spraying of narcissus Soleil d'Or with Bordeaux mixture in districts where *Sclerotinia polyblastis* is prevalent can be recommended as likely to give an increased yield without risk of delaying flowering.

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Varietal differences in susceptibility to potato virus *Y*

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In addition to giving different kinds of symptoms when infected with potato virus *Y*, individual potato varieties also differ in their susceptibility to infection, in the concentration of virus attained in their sap, and in their efficiency as sources of virus for aphides. Their relative susceptibility in the open when exposed to equal chances of infection is correlated with the ease with which they become infected when colonized with infective aphides, and can be assessed from tests made under glass. Methods for making such tests are described; these need few tubers and give reproducible results. It is considered that they could be applied in studying the inheritance of this type of resistance and to test the behaviour of new seedlings. The American variety Katahdin was the most resistant of those tested, but there were significant differences between commercial British varieties.

In the open, all varieties were equally colonized by aphides and resistance to infection with virus *Y* was not correlated with resistance to leaf roll.

From observations on commercial potato crops grown in the same districts, it is obvious that different varieties degenerate at different rates and often because of different virus diseases. In the south-east of England, for example, once-grown seed of the variety Arran Banner can usually be relied upon to give vigorous crops, whereas once-grown seed of Arran Pilot and Arran Consul cannot, because the Arran Pilot is likely to have a high proportion of plants with severe mosaic (potato virus *Y*) and the Arran Consul an equally high proportion with leaf roll. In spite of the practical importance of these differences, there is no evidence to show whether they are an indication of true varietal differences in susceptibility to individual viruses or a result of other factors such as selective colonization by aphides; it is possible even that the differences in health of once-grown seed produced in England merely magnifies differences existing in stocks of the different varieties as produced in the seed-growing regions. The last is unlikely, for field trials with leaf roll and severe mosaic in the U.S.A. (Schultz *et al.* 1937; Jones & Vincent, 1937; Jones & Burke, 1940; Stevenson *et al.* 1943) and in Germany (Kohler & Heinze, 1939), and with leaf roll in Ireland (Loughnane, 1941), showed that even when exposed to equal chances of contracting virus diseases some varieties did so more readily than others.

Few attempts have been made to study the basis of these varietal differences further, and these have failed, for varieties found to be resistant in field trials were readily infected by grafting or inoculation. The only exception was the variety Katahdin, which resisted infection with potato virus *Y* when rubbed with infective sap, and this resistance was found to be inherited (Jones & Burke, 1940).

We have made experiments with several different varieties, both in the open when exposed to natural conditions of spread and under glass with controlled conditions. The results have confirmed the differences observed in commercial crops and show clearly that there are real varietal differences in susceptibility to potato virus *Y*. Varieties differ in the ease with which they become infected and in the extent to which the virus multiplies once it has become established. Behaviour in the field is closely correlated with the former, and it seems that the result of transmission tests with aphides can be used as a reliable guide to the relative behaviour of different varieties in the field.

Field experiments

Small-scale field trials were made in which plots of different varieties were exposed equally to chances of infection. Each plot contained twenty-five plants, twenty-three of which were virus-free and two infected, one with leaf-roll virus and the other with potato virus *Y*. The plots consisted of five rows of five plants each, the one infected with virus *Y* being at the centre and the one with leaf roll adjacent to it. In 1942, trials were made at Rothamsted and at the Midland Agricultural College, Sutton Bonington, in which four such plots of each of six varieties were arranged in the form of an incomplete block. At the end of the growing season, a single tuber was taken from each of the originally healthy plants; these tubers were planted in 1943 and their health was recorded by examining the growing plants in July. The results (Table 1) show that there was a greater spread of both viruses at Rothamsted than at Sutton Bonington, but that the relative behaviour of the varieties at the two centres was similar. Some plants had both leaf roll and severe mosaic virus, but as the severe mosaic virus in such plants was sometimes difficult to diagnose with certainty these were recorded under leaf roll.

A similar experiment was made at Rothamsted in 1943, using the same six varieties plus Redskin and Katahdin. There was more spread of both leaf roll and severe mosaic than in the previous year, but the relative susceptibilities of the varieties was again the

same. Of the British varieties, Arran Banner and Majestic were most resistant, though 45% of these were infected, and Arran Pilot and May Queen least resistant with 90% infected. The most striking result was the great resistance of Katahdin. Only forty-six tubers of this variety were planted; when examined in July, forty-three of these had developed into healthy plants, one had leaf roll and the other two were missing. From the reactions of this variety, which are described below, it is probable that the last two tubers were infected with potato virus *Y*, but we have no definite evidence of this.

Periodic counts of aphides in the plants were made at Rothamsted in both years, but there was no significant difference between the infestation on different varieties. In 1942 there was a heavy infestation, ranging from 1600 aphides per 100 leaves on the Arran Consul to 2000 on the King Edward, but this

Table 1. *Relative susceptibility of potato varieties in the field to leaf roll and severe mosaic*

Variety	Percentage of plants in different categories: Healthy	Severe mosaic	Leaf roll
		Rothamsted	
Arran Banner	88	6	6
Majestic	83	7	10
King Edward	57	30	13
Arran Consul	37	13	50
May Queen	33	44	23
Arran Pilot	20	70	10
		Sutton Bonington	
Arran Banner	92	0	8
Majestic	94	2	4
King Edward	76	19	5
Arran Consul	65	0	35
May Queen	66	16	18
Arran Pilot	66	27	7

was almost entirely *Aphis rhamni*, and the figures for *Myzus persicae* over the whole trial never exceeded 20 per 100 leaves. In 1943 the infestation was light, but was mainly confined to *Myzus persicae*, which was uniformly distributed over the different varieties and at its peak reached 80 per 100 leaves. It is interesting to note that although *Aphis rhamni* is as efficient at transmitting potato virus *Y* as *Myzus persicae* in experimental conditions (Kassanis, 1942), it seems to have little or no effect on transmission in the open.

Glasshouse experiments

In the insect-proof glasshouses, comparative tests were made with different potato varieties on four properties that might explain their varying behaviour in the field. These were:

(1) Susceptibility to infection when rubbed with infective plant extracts.

(2) Susceptibility to infection when colonized with infective aphides.

(3) Virus concentration reached in infected plants.

(4) Efficiency as source of virus for feeding aphides.

We have evidence, which will be published later, that potato virus *Y* occurs in a number of strains which vary in the type of disease they cause in one and the same variety. In all these tests we used the same strain, a virulent one which in the first year of infection causes severe leaf-drop streak in the varieties Majestic, Arran Consul and Sharpe's Express. In other varieties this strain causes other diseases; in Arran Pilot, Ulster Monarch, Kerr's Pink and May Queen there is mosaic of various intensities with no necrosis; in Arran Banner symptoms are mainly a mosaic, but there is some venal necrosis and general chlorosis of lower leaves; in King Edward and Redskin mosaic predominates, but there is considerable necrosis and falling of the lower leaves; in Gladstone the main symptoms are necroses and these also appear in the second year of infection; in Katahdin the only symptoms are necroses which may be lethal, both in the first and second years of infection.

Infection by inoculation

When any of the British varieties we have used are rubbed with undiluted sap from either infected potato or tobacco plants, they become systemically infected and the only differences found are in the type of disease produced. The American variety Katahdin, however, shows considerable resistance, even by this crude method of testing. Occasional plants fail to develop any symptoms, but most show necrotic local lesions; some fail to develop systemic symptoms, and of those that do some become acutely necrotic and soon die, whereas others develop only scattered, isolated necrotic spots and stripes. Tubers from systemically infected plants give mixed results when used for seed purposes: some give healthy plants, some fail to grow and the remainder give acutely necrotic, minute plants which soon die. Systemic infection of this variety with potato virus *Y* is regularly obtained if carborundum or celite is incorporated in the inoculum.

Preliminary tests with the variety Majestic showed that the dilution end-point of sap from tobacco plants infected with virus *Y*, expressed when they are showing the secondary symptoms of vein-banding, usually lies between 1/200 and 1/400. In an attempt to refine the methods of testing, infective sap from such tobacco plants was used as inoculum after dilution to either 1/100 or 1/250 with water. In each experiment ten plants of each variety were inoculated when they were from 9 to 12 in. high, two leaflets of approximately the same size being rubbed as evenly as possible over their whole surfaces with the forefinger dipped in the inoculum. Under these conditions 100% infection was regularly obtained in tobacco plants, but not in potato varieties.

No clear-cut differences were found between the varieties, for there were great day-to-day variations between the results obtained, although in most experiments more infections were obtained in varieties such as Arran Pilot and May Queen, which react with mosaic symptoms, than in those such as Arran Consul and Gladstone, which produce local lesions and systemic necrosis. The only definite conclusions to be drawn from these inoculation tests were that tobacco is more easily infected than potato and that Katahdin is outstanding among potato varieties in its resistance to infection.

Infection by aphides

All tests were made with the aphis *Myzus persicae* Sulz. using the conditions found by Watson & Roberts (1939) to be optimal for transmission. The aphides were raised on turnips or radishes, and starved for 4 hr. before being used. They were then fed for 3–4 min. on the undersurface of an infected tobacco leaf and immediately transferred to the healthy test plants, which were fumigated 12 hr.

TABLE 2. *Effect of number of aphides in transmitting potato virus Y to different test plants*

Test plant	No. of plants infected out of 36 colonized with: 2 aphides per plant	4 aphides per plant
Arran Banner	3	10
Majestic	5	17
Arran Consul	10	19
Arran Pilot	12	25
May Queen	15	21
Tobacco	25	36

later. Potatoes were used as test plants when they were between 3 and 5 in. high. It seems that within fairly wide limits the age of the test plant is immaterial, for in one experiment with the variety Majestic, three lots of twenty plants, one the usual age and the others 15 and 30 days older, gave eleven, nine and thirteen infected plants respectively, when five aphides were used per plant.

In 1942 preliminary tests were made with five potato varieties and tobacco to determine both the best number of aphides to use per plant and the reproducibility of results. Six experiments were made, in each of which twelve plants of each variety were used, half the plants being colonized with two aphides each and the other half with four aphides. The results of different experiments agreed with one another closely and showed that there were clear varietal differences in susceptibility (Table 2), which reflected the behaviour in field trials.

Greater differences were obtained between different varieties when two aphides were used than with four, so in 1943, when larger experiments were made with more varieties, two aphides were always used. Five experiments were made at different times with thirteen potato varieties and tobacco, using ten plants of each variety on each occasion. The results from different experiments agreed well with one another, but to test their homogeneity the χ^2 test was applied. This showed that there was no significant difference between the results obtained at different times, so that they could legitimately be totalled. The proportion of infected plants was treated as a percentage and transformed into angular degrees, from which standard errors were calculated. The results are given in Table 3 and show that there are significant differences in the behaviour of the different test plants. At one end of the scale is the American variety Katahdin and at the other the variety Ulster Monarch, which is nearly as susceptible as tobacco. Most of the common British varieties tested were intermediate in their behaviour and fall into two

TABLE 3. *Relative susceptibility of potato varieties to virus Y transmitted by* Myzus persicae *Sulz.*

Variety	% test plants infected	Angular degrees	Standard error
Tobacco (White Burley)	76	60·7	±4·0
Ulster Monarch	70	56·8	±5·3
May Queen	52	46·1	±4·1
King Edward	52	46·1	±4·1
Arran Pilot	50	45·0	±3·9
Arran Consul	36	36·9	±4·0
Kerr's Pink	28	31·9	±4·0
New Seedling (T.H.)	26	30·7	±4·9
Gladstone	24	29·3	±4·0
Sharpe's Express	24	29·3	±4·0
Redskin	22	28·0	±4·9
Arran Banner	18	25·1	±4·0
Majestic	16	23·6	±4·0
Katahdin	8	16·4	±4·0

groups, one giving from 15–30% infected plants with the method of testing and the other from 50–60%.

The concentration of virus in different varieties and its availability to Myzus persicae

Most spread of potato virus diseases is over short distances, and healthy plants are more likely to contract virus *Y* from infected plants in their immediate vicinity than from elsewhere. Except for groundkeepers, such healthy and infected plants are likely to be of the same variety, so that if aphides more readily become infective when feeding on one variety than on another, this could also be a factor in determining the rate of degeneration. To test this we have made experiments with the varieties Arran Banner, Arran Pilot, May Queen and Majestic, in both their first and second years of infection with virus *Y*. Previously starved aphides were fed for

3 min. on the undersurfaces of infected leaves, showing mosaic symptoms only, and then transferred to a standard test plant, healthy tobacco seedlings. Two aphides were used for each test plant, and in each experiment ten test plants were used for each variety. The experiment was repeated four times with leaves from potatoes in their first year of infection and seven times with plants raised from infected tubers. As the ability of aphides to become infective in a given time of feeding might be expected to depend on the concentration of virus in the diseased leaves, this was also measured serologically on each occasion. Samples of the leaves on which the aphides fed were minced, the sap expressed and clarified by centrifuging for 15 min. at 10,000 r.p.m. The supernatant fluid was then titrated against antiserum to virus *Y* used at a constant dilution of 1 : 50. The greatest dilution at which a precipitate was obtained after incubation for 1 hr. at 35° C. was recorded as the precipitin titre. The results in Table 4 show that aphides become infective more readily when feeding on some infected plants than on others, and that, except for the variety May Queen in the first year of infection, the results of the transmission tests are closely correlated with the virus content as shown by the precipitin titre.

TABLE 4. *The relationship between virus content of different varieties and aphis transmission from them to tobacco*

	Plants in first year of infection		Plants in second year of infection	
Source of virus	% test plants infected	Virus content*	% test plants infected	Virus content*
Tobacco	80	12	—	—
Arran Banner	55	5	60	5·5
May Queen	45	2·5	55	5·5
Arran Pilot	43	3·5	44	4·5
Majestic	8	0·25	24	2·5

* Virus content expressed as the reciprocal of the average precipitin titre. Description of methods in text.

The results of these tests have a bearing on those given in Table 3, which were interpreted as suggesting that different varieties differ in the initial dose of virus needed to cause infection. An alternative possibility, however, was that varieties differ in the readiness with which aphides become infective when fed on them. Table 4 shows that this is unlikely, for aphides fed on infected Arran Banner, one of the varieties most resistant to infection by aphides, gave more infections in tobacco than those fed on other varieties, which are more easily infected. The results also show that the multiplication of the virus once it has become established in a plant is not necessarily correlated with susceptibility to infection. Of the two British potato varieties most resistant to infection with potato virus *Y* when transmitted by aphides, one, Arran Banner, develops a high virus concentration and is a favourable source of virus for aphides, whereas the other, Majestic, develops a low virus concentration and is an unfavourable source of virus for aphides.

DISCUSSION

In breeding for disease resistance the ideal objective is an immune variety. There is as yet little hope of this being achieved with potatoes and virus diseases, because, except for the American seedling U.S.D.A. 41956, which is immune from all strains of virus *X* yet tested, parents with the requisite genes are unknown. In the absence of immunity, however, there are other characters that can be employed to reduce losses. The character most used so far is hypersensitivity, and varieties are known which give local lesions only or which die with a systemic necrosis when infected with viruses *A*, *B*, *C* and *X*, and the variety Craig's Defiance has been bred which is hypersensitive to all four (Cockerham, 1943).

Unfortunately, no variety is known that is hypersensitive to leaf roll and potato virus *Y*, the two viruses responsible for degeneration in most parts of Britain. However, the behaviour of Katahdin towards potato virus *Y* approximates to hypersensitivity, for infected plants react so severely that it is unlikely they would persist long in a stock as a source of infection for neighbouring healthy plants. Katahdin also has another desirable character more strongly developed than in any other variety we have tested, namely, resistance to initial infection with virus *Y*. This character has not as yet been much studied, possibly because the difficulties of testing for it in the early stages of raising seedlings have not been overcome. Most potato breeding is naturally done in districts where aphides are rare, and it is usually not until varieties have been in commerce for some time that there is any indication of their resistance or susceptibility to the common virus diseases. Katahdin is clearly the most suitable variety for use as a parent in breeding for resistance to potato virus *Y*, but our results also show that British varieties differ in the extent to which they become infected when exposed to the same conditions. These differences are sufficiently large to be of economic importance and to explain, for example, why once-grown seed of Arran Banner usually gives a reasonable yield, whereas once-grown seed of Arran Pilot produced in the same conditions does not.

To study the inheritance of varietal differences in susceptibility, tests are needed which can be applied early in the life of a seedling. The results of our small-scale field trials suggest that, provided they are made in districts where leaf roll and potato *Y* viruses spread fairly rapidly, tests of susceptibility com-

paring established varieties and new seedlings could be made reliably with 100 sets only. The glasshouse tests using aphides in standardized conditions could be done even earlier in the life of a seedling, for there would probably be ample material at the end of the second year. This method would certainly be valuable for selecting suitable parents and for rejecting very susceptible seedlings, but because of the existence of strains of viruses and the frequency with which new strains seem to occur, glasshouse tests will always need supplementing by field tests. In our work we have used only one source of potato virus *Y*, and it is possible that other sources would behave differently; with some of these the variety Katahdin may react differently and prove much easier to infect.

Our field tests show that susceptibility to infection with virus *Y* is independent of susceptibility to leaf-roll virus, for when exposed to equal chances of infection with both viruses, some varieties became mainly infected with *Y* and others with leaf roll. The reasons for these varietal differences in susceptibility are unknown, but the simplest explanation is that there is a minimal quantity of virus necessary for infection to occur and this varies with different varieties.

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Resolution of strawberry virus complexes by means of the aphis vector *Capitophorus fragariae* Theob.

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(With Plate 4 and 1 Text-figure)

Aphides (*Capitophorus fragariae*) were fed for periods of up to 24 hr. on strawberry plants infected with mild crinkle, severe crinkle or yellow-edge and then transferred to plants of the wild strawberry, *Fragaria vesca*, or of the cultivated strawberry, variety Royal Sovereign. On *F. vesca* the symptoms produced were chlorotic speckling, distortion and dwarfing of the leaves, varying in intensity, and on Royal Sovereign scattered, inconspicuous, diffuse, chlorotic spots.

The symptoms from all three sources of infection were similar and were indistinguishable from those of mild crinkle of Harris & King. The virus thus selectively transmitted is tentatively concluded to be the mild crinkle virus.

The virus was transmitted after feeding periods of 1 hr. or more and did not generally persist in the vector for more than 3 hr.

Introduction

The variation in severity of the symptoms of crinkle (Zeller & Vaughan, 1932; Ogilvie *et al.* 1934) and of yellow-edge (Harris, 1933) has given rise to the suggestion that these diseases may be caused, not by single viruses, but by mixtures or complexes of viruses or of virus strains (Zeller, 1933; Harris, 1937; King & Harris, 1942). The frequent occurrence of crinkle in association with yellow-edge or with xanthosis (which is perhaps identical with yellow-

edge) has also been noted (Zeller, 1936; Harris & King, 1942), but it is not known whether this association is obligate or fortuitous.

A method of separating the component viruses from mixtures occurring in strawberry plants would obviously help to clear up such points but, while there are several well-known methods of separating viruses from mixtures, most of them are directly applicable to sap-transmissible viruses only. Other methods depend on the fact that the viruses to be separated are transmitted by different means or possess different host ranges or that one virus is transmitted by an insect which does not transmit the other. The strawberry viruses are not sap-transmissible (Harris & King, 1940) and do not appear to be present in expressed sap (Bawden & Kleczkowski, 1945); they have few known vectors (Whitehead & Wood, 1941) and their known host range is very restricted. Such methods of separation are therefore not applicable.

A method making use of the different relationships of the potato viruses *Y* and leaf-roll to the vector *Myzus persicae* has been employed to separate these viruses (Bawden, 1943, p. 81). This method can be extended, since it has been shown that, for 'non-persistent' viruses, the efficiency of the vector is increased by preliminary fasting and short infection feeding (Watson, 1938). When aphides subjected to preliminary fasting are fed for short periods on sugar-beet infected with yellows and mosaic, they usually transmit mosaic alone when transferred to healthy plants; when long feeding periods are given, the majority of the transmissions are of yellows alone (Watson, 1945).

Preliminary experiments (begun in 1942) in the separation of the elements of strawberry virus complexes utilizing differences in vector relationships are described below.

Experimental

Aphides (*Capitophorus fragariae* Theob.) from a stock raised on cultivated or wild strawberry plants (which showed no symptoms and were believed to be virus-free) were transferred by means of a moistened camel-hair brush to one-half of a Petri dish. The Petri dish contained a piece of moistened filter paper so as to maintain a humid atmosphere and was tightly covered by a piece of Cellophane held in position by a rubber band. The aphides were inserted through a small hole in the Cellophane, the hole being sealed later by a moistened patch of the same material.

Aphides were allowed to fast in the Petri dish for about 18 hr. and were then transferred to detached leaves of infected plants. The leaves, whose petioles were embedded in moist sand, were from strawberry plants (var. Royal Sovereign) infected with mild crinkle, severe crinkle or yellow-edge and aphides were left on the leaves for periods of 5 min., 1 hr. and 24 hr. It was observed that aphides took about 3 min. to assume a feeding position and thus aphides left on the leaves for 5 min. probably fed for about 2 min. After being allowed to feed for these periods, two aphides were transferred to each of a number of young virus-free plants of *Fragaria vesca* and subsequently to three other series of *F. vesca* plants (Text-fig. 1). All *F. vesca* plants were kept in the glasshouse and sprayed weekly with an insecticide wash.

No symptoms appeared on any of the *F. vesca* plants receiving aphides fed for 2 min. or on any of the control plants receiving aphides direct from the stock, and only one of the sixty plants receiving aphides fed for 1 hr. became infected. Plants infected by aphides receiving a 24 hr. infection feed are indicated in Table 1.

A similar series of experiments was conducted using young strawberry plants of the cultivated hybrid variety Royal Sovereign ('Malling 40' clone) as indicators, but, owing to the faint and indefinite symptoms produced, it was not possible to diagnose infection on this variety. Royal Sovereign plants, whether infected by direct aphis transfer or by grafting to infected *F. vesca* plants, developed only very slight chlorotic spotting and sometimes showed no symptoms at all, so that infection had to be confirmed by grafting to healthy *F. vesca*.

Table 1. Fragaria vesca *indicators infected by aphides receiving an infection feed of 24 hr.*

Source of infection	Proportion of indicators developing symptoms* 1st transfer (10 min.) 3*A*†	2nd transfer (2 hr.) 3*B*†	3rd transfer (24 hr.) 3*C*†	4th transfer (24 hr.) 3*D*†
Yellow-edge	2/5	3/5	1/5	0/5
Severe crinkle	2/5	5/5	3/5	0/5
Mild crinkle	0/5	4/5	1/5	0/5
Total	4/15	12/15	5/15	0/15

* Numerators show number of plants infected; denominators show number of plants colonized with aphides.

† See Text-fig. 1.

Symptoms on *F. vesca* took about 20–22 days to appear and, as with Royal Sovereign, were of the same general type, no matter whether the source of infection was a plant infected with yellow-edge, severe crinkle or mild crinkle. Angular chlorotic flecks appeared on the leaves, accompanied by puckering or 'blistering' and distortion of the leaf and reduction in the size of the lamina (see Plate 4, Figs. 1, 2). Occasionally slight clearing of the leaf veins was noted as a preliminary symptom. The symptoms, however, varied in severity and it seemed that they could be classified as mild (Fig. 1) or severe (Fig. 2). Attempts to differentiate the causal viruses on

grounds other than symptomatology have not, so far, given positive results, and it may be that the variation in severity is caused by individual plant reaction or by the existence of two or more strains of one virus.

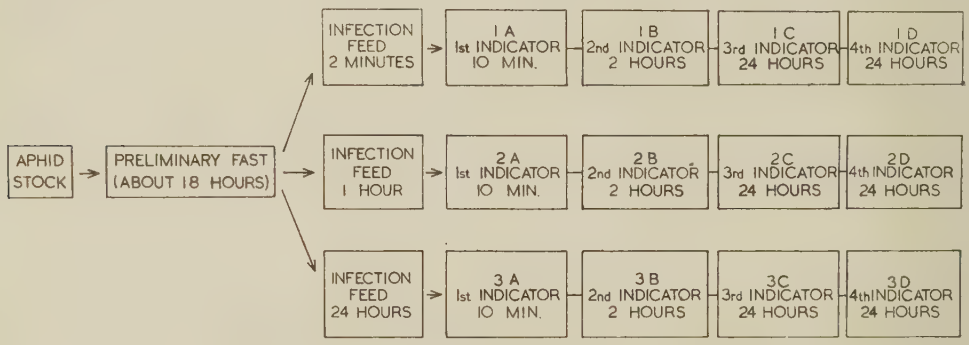
Such a variation in the severity of symptoms on *F. vesca* infected with crinkle has already been noted and the two main symptom types have been figured (Harris & King, 1942). In the present experiments, while both types occurred on plants infected from severe crinkle and yellow-edge sources, only the mild symptom type was transmitted from the plant infected with mild crinkle; in grafting experiments both symptom types have been reported from mild crinkle sources (Harris & King, 1942). It may be that the particular mild crinkle infected plant used as infector in the present experiments was infected with the mild symptom type only.

Both the mild and severe symptom types are transmitted occasionally by aphides which have fed on an infected leaf for 1 hr., but a much larger proportion of infections is produced by aphides fed for 24 hr. Combined data for the mild and severe symptom types are presented in Table 2. Each figure is a total from a number of experiments, from one to ten aphides per indicator being employed in different experiments. Larger numbers of aphides were used in experiments with short feeding times than with long feeding times, so that the increased 'efficiency' with longer feeding periods is really even greater than would appear from an examination of this table. Small-scale comparative trials suggested that fasting had no effect on the ability of the vector to transmit infection, and in most of the experiments summarized below, aphides were not subjected to preliminary fasting.

It was found that aphides which have fed on an infected leaf for 24 hr. lose their infectivity within about 3 hr. From Table 1 it will be seen that there were no infections among plants of the fourth transfer, and thus the virus does not persist in the vector for more than about 24 hr. In other experiments only one plant out of seventeen was infected by aphides which had been removed from the source of infection for 3 hr. In one case, after aphides had fed on an infected leaf for 24 hr., five of them were transferred to each of eight plants for 3 hr. and then retransferred to eight fresh plants for 21 hr. Seven of the plants of the first (3 hr.) transfer became infected and none of those of the second (21 hr.) transfer. In a second experiment, two aphides after a 24 hr. infection feed were transferred to each of ten *F. vesca* plants for 1¼ hr., retransferred for ¾ hr. and again retransferred for 1 hr.; seven plants of the first series, one of the second and none of the third became infected. In a third experiment, two aphides after a 24 hr. infection feed were serially transferred for ¼, ¼, ¼ and 2¼ hr. to sets of ten plants; three, five, two and two plants respectively, became infected. It would thus appear that infectivity begins to fall off within an hour and is practically nil 3 hr. after leaving the source of infection.

Table 2. *Effect of length of infection feed on transmission of the mild crinkle virus*

Infection feeding period	Proportion of infections	Percentage
2 min.	0/50	0
1 hr.	5/70	7
4 hr.	9/50	18
24 hr.	33/50	66
40 hr.	13/15	87



Text-fig. 1. Scheme of aphis transfers in a typical experiment.

Discussion

Assuming that the mild and severe symptoms on *F. vesca* are caused by plant variation or by strains of one virus (and this explanation is supported by the similarity of the relationships of the vector to the causal viruses), it is tentatively concluded that

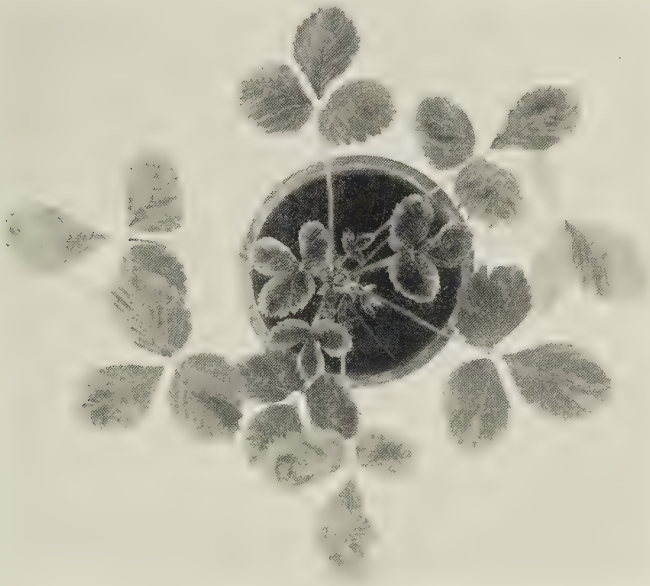


Fig. 1

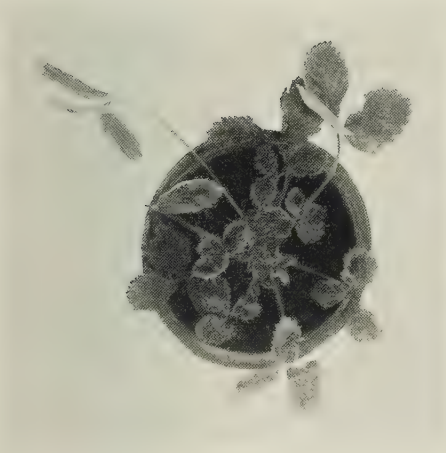


Fig. 2

Fig. 3

one virus has been isolated from plants infected with mild crinkle, severe crinkle and yellow-edge. The symptoms produced on both *F. vesca* and Royal Sovereign closely resemble those of mild crinkle, and it is thought that the isolated virus is the mild crinkle virus. It has been shown (Massee, 1935, 1942) that *Capitophorus fragariae*, the aphis used in the present experiments, is a vector of yellow-edge and severe crinkle, and it is probable that failure to transmit these symptoms is due to the conditions of the present experiments, for example, the relative shortness of the feeding periods employed.

The isolated virus (mild crinkle virus) is occasionally transmitted after infection feeds of 1 hr., but the proportion of infections increases with increasing infection feeding time. Prefasting of the aphides appears to have no effect on transmission. Thus, although the mild crinkle virus does not persist in the vector, it fails to conform to the characteristics of a 'non-persistent' virus since viruses of this type are more readily transmitted after fasting and short infection feeding (Watson & Roberts, 1939; Watson, 1938). The vector relations of the mild crinkle virus appear, however, to resemble those of dandelion yellow mosaic (Kassanis, 1944).

Although the presence of the mild crinkle virus in plants infected with severe crinkle and yellow-edge has been confirmed, it is still not clear whether it is an essential constituent of complexes producing these diseases or whether its occurrence in association with them is purely fortuitous. Isolation of other viruses from infected strawberry plants and resynthesis of severe crinkle and yellow-edge is necessary to elucidate this and work on these lines is in progress.

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EXPLANATION OF PLATE 4

Fig. 1. Infected plant of *Fragaria vesca* showing mild symptoms.
Fig. 2. Infected plant of *F. vesca* showing severe symptoms.
Fig. 3. Normal plant of *F. vesca*.

Approx. $\frac{1}{3}$ natural size.

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Field trials of copper fungicides for the control of potato blight

II. Spray retention

By E. C. Large, B.Sc., A.R.I.C., *Seale-Hayne Agricultural College, Newton Abbot, Devon*, W. J. Beer and J. B. E. Patterson, M.Sc., F.R.I.C., *The Laboratory, Dartington Hall Trustees, Dartington, Devon*

(With 2 Text-figures)

Spray-retention estimations were made during four seasons at Dartington in south Devon, on maincrop potatoes, twice-sprayed, as in farm practice, with about 120 gal./acre for the first application and 160 gal. for the second.

Evidence is given concerning the limits of reliability of the disk method of leaf sampling; and an alternative method, battery washing, is described.

With from 2½ to 3½ in. of rain over test periods of 3–4 weeks, 1% Bordeaux mixture showed about 40% spray retention. Cuprous oxide and copper oxychloride sprays at the same copper dosage but compounded with sulphite lye or other water-soluble dispersing agent showed less than 20% retention. Compounding of these materials with an insoluble sticker (bentonite) improved the adhesion.

The percentage retention of ½% Bordeaux mixture was found to be less than that of the usual 1% mixture, but adequate spray deposits could be maintained with this and other low-copper fungicides by more frequent applications.

A rapid method for determining total expanse of foliage in the field is described, and the importance of such determinations in connexion with spray-retention trials, and practical spray timing, is stressed.

Experiments were made to determine whether any copper is absorbed by potato leaves from the spray deposits. Absorption, or acid-resistant adsorption, of the order of 0·02 mg. Cu/120 sq.cm. of leaf area was indicated.

Introduction

The principal published work on the field testing of potato-spray retention in the British Isles, at the inception of the present trials, was that begun by Martin (1933) at Wye, in Kent, and continued at Long Ashton, near Bristol, by Marsh *et al.* (1937), Fajans & Martin (1937, 1938) and Marsh & Martin (1940). In most of the trials made near Bristol potato blight was either absent or very slight, and for the particular purposes of the work then in hand, the spray fluids were applied at the rate of 300–400 gal./acre.

Our aims in the present work were:

(*a*) To carry out similar trials, also over a period of years, and with a number of differing copper fungicides, but at normal rates of application (100–160 gal./acre) and in a district of higher rainfall.

(*b*) To obtain spray-retention data that could be correlated with estimates of foliage protection and yield, year by year, in spraying trials made in a locality, to the south-east of Dartmoor, where potato blight is usually fairly severe.

(*c*) To develop the existing methods of field testing, by the use of a more sensitive method of copper estimation; by further study of sampling errors; and by devising alternative means of checking the results obtained.

The work on foliage protection and yield has been dealt with in a previous paper (Large, 1945), to which reference should be made for full particulars of the lay-out of the trials and of the spray materials employed.

Method of copper estimation

Except where otherwise stated, all copper estimations were made on samples of fifty leaf disks, 17·5 mm. diam. (area 120 sq.cm. or 18·6 sq.in.) freshly cut with a cork-borer from packs of fifty leaflets, and ejected into cleaned 3 × 1 in. tubes on the field. The method of copper estimation, using the diethyldithiocarbamate colour reaction, was based on that of Steussij (1938). To each of the tubes containing the leaf disks, 5 ml. conc. nitric acid was added. After standing overnight 5 ml. conc. sulphuric acid was applied, and the whole was then washed into a 100 ml. Kjeldahl flask by the use of about 50 ml. of *glass-distilled* water. It was found of importance that all the distilled water used should be redistilled from resistance glass. The contents of the flask were heated, at first gently until the water had evaporated, and then more strongly until the nitric acid was driven off and SO_3 fuming had continued for about 1 hr. If the solution after digestion had a yellow tint, as was usual when the sample had a relatively high copper content, a further 5 ml. of nitric acid was added and the process was repeated.

The cooled solution was diluted with 20 ml. glass-distilled water, recooled, and washed into a 100 ml. measuring flask and made up to the mark. The solution was then filtered through a fluted Whatman no. 2 9 cm. paper into a dry 100 ml. conical flask: 25 ml. of this final solution was then pipetted into a 50 ml. measuring flask and 5 ml. of 2% gum arabic solution was added with stirring, followed by 1 ml. of 1% aqueous sodium diethyldithiocarbamate, freshly made up for each day's work and filtered through a Whatman no. 42 paper.* The flask was shaken immediately after addition of the reagent and the contents made up to the mark with glass-distilled water.

The colour density of the solution after standing for 15 min. was read by a Spekker absorptiometer, using 1 cm. cells and a no. 6 colour filter (blue-green). The Spekker reading was converted to equivalent copper concentration by reference to a standard curve, obtained from standard solutions containing 0·01–1·0 mg. Cu/50 ml. with 2·5% sulphuric acid. The standard curve was reproducible with great accuracy from freshly made-up standard solution, and it was found necessary to check only one or two points on the curve before each set of determinations.

The strength of the solutions could be read to within ±0·005 mg. Cu/50 ml. (equivalent to ±0·02 mg. Cu/50-disk sample). The sensitivity of the method was such that it would detect differences of 1 part of copper in 10 million parts of solution.

Sampling method

(1) *Location of sample area on leaflets*

In a pilot trial in 1942 on plants in a greenhouse, leaf disks were cut as shown in Fig. 1, to compare the amount of copper accumulated at the leaf tips due to drainage or run-off, with that more or less uniformly distributed over the rest of the leaflet area. Blocks of well-grown plants (var. Great Scot) were sprayed as evenly as possible on 8 May, (*a*) with 1% Bordeaux mixture, which tends to give a close spattering of deposits over the leaf surface without much spread; (*b*) with a cuprous-oxide preparation (Perenox) which runs more freely; and (*c*) with a mixture of red cuprous oxide (3 parts) and sodium alginate (Manucol V) (1 part). The object of the last-named treatment was to try the effect of a spreader and sticker of high viscosity. All the sprays were applied at the same copper dosage (0·25% Cu) and at about the same rate as in the field. Fifty leaflets were collected from each block of plants as soon as the spray deposits had dried, and two samples of 50 disks, 17·5 mm. diam., were cut from the central portions of the leaflets, and one sample of 50 disks 10 mm. diam. was cut as close as possible to the leaflet tips. Similar samples were also taken from an unsprayed block.

The plants were then subjected to 'artificial rain' by watering each day with a fine rose for a period of 33 days. A rain gauge standing among the plants showed that the amount of water so applied was equivalent to about 5·75 in. of rain (0·15 in. at each watering). At the end of the test period, on 10 June, the foliage was sampled as before, with care to see that none of the new growth, not sprayed on 8 May, was included. The results obtained are shown in Table 1.

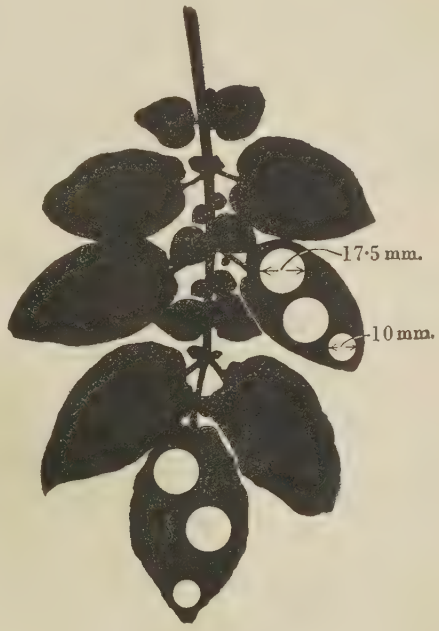

Fig. 1. Leaf sampling. In comparisons of deposits on centre of leaflets and on leaf tips, disk samples were cut as shown. In the main trials (Table 3), one 17·5 mm. disk was cut from the centre of each sample leaflet. By the battery-washing method (Table 7) a mean value was obtained for the copper coverage over the whole compound leaf.

The copper deposits on the tips of the leaflets were from twice to four times as heavy as on the central parts, with an indication that there was less run-off or drainage to the tips with Bordeaux mixture and the high viscosity spray than with the more free-running

* Steussij (1938) and others have used sodium pyrophosphate to neutralize the colour effects of iron, but this was found to be unnecessary and undesirable in the present work, as the coloration due to very small amounts of iron in potato leaves was negligible, and the addition of sodium pyrophosphate caused turbidity where Bordeaux mixture had been employed, owing to the formation of calcium pyrophosphate. Concentration of the carbamate complex colour by extraction with amyl alcohol was also found to be unnecessary.

Perenox. For the main trials, which included materials of differing physical characteristics, it was therefore decided to cut the leaf disks from the central portions of the leaflets, as Martin and his co-workers (1937, 1938, 1940) had done in their trials, and, later, to compare the results so obtained with those from trials in which the whole of the copper on the leaves would be removed by a battery-washing method, to be described below.

(2) *Collection of leaf samples*

Fajans & Martin (1937) collected twenty-five leaflets at random from each of their replicated plots, and then mixed all the twenty-five leaflet samples for each treatment in the laboratory. From this single bulked sample they then cut four lots of fifty leaf disks (1·325 cm. diam.) which they ashed and estimated separately. They adopted a similar procedure in other years, and the statistical examinations of their results showed throughout merely the variation in copper content between different portions of a single bulked sample.

TABLE 1. *Spray deposits on centre of leaflets and on leaf tips*

(0·01 mg. Cu/129 sq.cm.)

	8 May		10 June	
	Centre	Tips	Centre	Tips
Bordeaux mixture	164		101	
	118	251	115	139
Perenox	119		57	
	109	408	76	190
Red cuprous oxide with sodium alginate	121		50	
	137	277	56	150
Unsprayed	4		3	
	4	4	1	5

In the present work we desired to explore the variation in the amount of copper deposit actually occurring over an apparently uniformly sprayed area, and so to ascertain how representative our samples really were of all the foliage subjected to a given spraying treatment. For this reason we increased both the number and the size of the disk samples taken per plot from twenty-five of 13·25 or 14·15 mm. diam. to fifty of 17·5 mm. diam. (thus increasing the area about three times), and *we did not bulk our samples* but made separate estimations for each plot.

In taking the samples, the method we adopted was to walk between the rows, plucking leaflets at random from about mid-height of the plants, avoiding both old leaves at the base and new growth at the top. The leaflets were taken at more or less uniform intervals, i.e. about every step, along the entire length of the rows in two traverses of each plot. Immediately the fiftieth leaflet was plucked, the whole wad of leaflets was pressed on to a rubber bung, and the cork borer was thrust once through it, avoiding both leaflet edges and tips. The pile of fifty disks was ejected directly into one of the previously cleaned glass tubes, which was then at once corked and labelled.

Besides providing independent samples from each plot, this method ensured that they were cut while the leaves were perfectly fresh, without disturbance of the spray deposits; and the method was also economical of time and labour when sampling a large number of plots, as all the necessary tubes could be carried in a haversack.

In each of the three main trials, in 1942, 1943 and 1944, samples were taken on four occasions: just after the first spraying, before and after the second spraying, and about 3 weeks after the second spraying.

(3) *Sampling error*

During the trial in 1943, six independent samples of fifty disks were taken on each sampling occasion from a single one of the plots sprayed with 1% Bordeaux mixture. The copper contents of the samples are given in Table 2.

TABLE 2. *Variations in amount of copper found in six leaf samples from a single uniformly sprayed plot*

(0·01 mg. Cu/120 sq.cm.)

Rain 5·18 in.		Rain 1·92 in.	
7 July	6 Aug.	7 Aug.	27 Aug.
122	27	170	88
111	20	200	79
140	24	171	84
98	25	224	116
129	35	198	80
116	27	151	76
119±6	26±2	186±11	87±6

On each sampling occasion the standard error was within ±8% of the mean value found, and there was a clear indication that in attempts to measure so intrinsically variable a quantity as the distribution of spray deposits over foliage in the field, no closer agreement was to be expected.

In the main trials (Table 3), in which independent samples were taken from each of the several replicated plots for each treatment, the standard errors of the means were obtained by analysis of variance, and it will be seen that they were within ± 10% of the mean values, with four or six independent samples. The measure of variation thus obtained could not be provided by bulk sampling.

COMPARATIVE FIELD TRIALS

Comparative estimates of spray retention by the disk sampling method were made for all treatments in the four field trials at Dartington described in a previous paper (Large, 1945).

In 1941 the sprays were applied on single 0·4 acre plots of Arran Consul potatoes with a horse-drawn

sprayer, at the rate of about 100 gal./acre for both the first and the second applications. Samples of 100 standard disks were taken after the second spraying only.

In 1942, ten treatments and four controls were randomized in three blocks of fourteen plots each 20 yd. long by six rows wide, and the spray fluids were applied uniformly at the rate of 120 gal./acre for the first application, and 160 gal. for the second, with a knapsack sprayer. One fifty-disk sample was taken from each plot on each sampling occasion. Variety: Majestic.

In 1943, twelve treatments and four controls were randomized in three blocks of sixteen plots each 15 yd. long by four rows wide, and all the sprays were applied at 160 gal./acre on both occasions. Two independent fifty-disk samples were taken from each plot on each sampling occasion. Variety: Majestic.

In 1944, seven treatments and one control were randomized in four blocks of eight plots each 27 yd. long by four rows wide, and all the sprays were applied at the rate of 80 gal./acre for the first application (when the expanse of foliage was small, vide Fig. 2), and at 160 gal./acre for the second application. One fifty-disk sample was taken from each plot on each sampling occasion. Variety: Majestic.

The mean copper estimates obtained in all the trials are assembled in Table 3, together with dates of spraying and sampling, and rainfall over each test period.

The figures for initial spray retention on 11 July and 14 Aug. 1942 were somewhat high for the copper dosages applied, as the plants were smaller than usual on the dates of spraying. In 1943 there was about 0·1 in. of rain between the first spraying on 5 July and the sampling on 7 July, with the result that some of the initial deposits, especially from the less retentive sprays, were washed off before sampling. In 1944 an abnormally high initial deposit in some of the Bordeaux plots was due to trouble with the sprayer. Apart from these explainable irregularities, the differences between the amounts of copper initially deposited on the leaves, with different sprays at the same copper dosage, were at most barely

TABLE 3. *Mean copper content of leaf samples in field trials by disk method*

(0·01 mg. Cu/120 sq.cm.)

Year ...		1941		1942				1943				1944			
Spraying date ...	...	31 July		10 July		13 Aug.		5 July		6 Aug.		29 June		27 July	
Sampling date ...	...	1 Aug.	28 Aug.	11 July	11 Aug.	14 Aug.	7 Sept.	7 July	6 Aug.	7 Aug.	27 Aug.	29 June	24 July	25 July	23 Aug.
Rainfall, in.		5·0		2·52		3·26		5·18		1·92		3·94		3·23	
No. of samples		1	1	3	3	3	3	6	6	6	6	4	4	4	4
Treatment	% Cu														
½B×6* ½% Bordeaux	0·125	—	—	—	—	—	—	—	—	—	—	67	69	82	39
B 1% Bordeaux	0·25	191	82	138	63	242	118	105	22	176	75	186	53	138	63
D Cuprous oxide with bentonite	0·25	—	—	153	34	273	195	104	15	83	24	117	26	142	76
½B ½% Bordeaux	0·125	—	—	78	23	150	51	—	—	—	—	72	10	59	29
½BX ½% Bordeaux with excess lime	0·125	—	—	—	—	—	—	73	12	116	36	52	16	54	24
½D Cuprous oxide with bentonite	0·25	—	—	—	—	—	—	62	9	70	17	—	—	—	—
J Jeunite	0·25	165	64	116	15	222	27	—	—	—	—	—	—	—	—
KB Potash Burgundy	0·25	—	—	—	—	—	—	—	—	—	—	—	—	154	62
K Metallic copper with bentonite	0·25	—	—	154	25	216	39	113	13	100	30	—	—	—	—
M Copper oxychloride with bentonite	0·25	—	—	122	34	115	48	—	—	—	—	—	—	—	—
C Coppesan	0·25	144	17	178	16	225	39	87	11	148	27	—	—	—	—
P Perenox	0·25	132	19	168	26	247	63	97	15	150	35	105	13	92	19
S Soltosan	0·25	110	12	111	21	136	34	—	—	—	—	—	—	—	—
H Metallic copper with sulphite lye	0·25	—	—	107	15	136	35	—	—	—	—	—	—	—	—
Unsprayed		5	3	9	6	10	6	5	4	5	4	5	4	4	4
S.E. (B to H)				±15	±5	±30	±12	±9	±1½	±10	±4½	±8	±4	±6	±8
Significant difference ($P=0·05$)				44	15	86	35	26	4	28	13	23	11	19	23

* Six applications of ½% Bordeaux mixture on 29 June, 12 July, 24 July, 11 Aug., 23 Aug. and 11 Sept. All other treatments two sprayings only, on dates given at head of table. For full particulars of treatments see p. 321 of previous paper (Large, 1945).

significant, and the deposits from sprays used at half strength were about half of those used at full strength. The rather greater tendency of the more free-running spray fluids to drain to the tips and to run off the leaves made no practical difference to the amount of copper drying on the centre parts of the leaflets.

The critical figures for spray performance, however, are those showing the amounts of copper still retained on the leaves after the seven test periods of from 3 to 4 weeks with 2½–5 in. of rain. Here, as would be expected, the amount of copper remaining after the wetter periods was generally less than after the drier, with variations attributable to differences in the distribution of the rain over the periods, as well as to its total amount. But in all the trials one point was clearly brought out: the amount of copper remaining on the leaves 3–4 weeks after spraying with 1% Bordeaux mixture was consistently and significantly greater than that left from all the other sprays except D, applied at the same copper dosage. The mean figures for all periods in 1942 and 1943 (in which all the treatments were included) are chosen as fairly indicative of the general performance of the sprays, as regards spray retention, in all the trials. It is considered that the limits of error in the sampling justify the placing of the treatments into two groups, the first with spray retention about twice that of the second, but with cuprous oxide and bentonite, and ½% Bordeaux mixtures, occupying an intermediate position.

Low-copper Fungicides

In the 1943 trial the treatments included three low-copper fungicides, applied twice only, at the same times as the sprays of normal copper dosage. The results are shown in Table 5.

Except with the ¼% Bordeaux mixture, the total amounts of copper found in the leaf samples taken at the ends of the trial periods were not significantly

Table 4. *Percentage retention of spray deposits*

		1941	1942		1943		1944		Mean (1942, 1943)
	Trial period ...	2	1	2	1	2	1	2	
	Rain, in. ...	5·0	2·5	3·3	5·2	1·9	3·9	3·2	
1	1% Bordeaux mixture (B) 0·25% Cu	42	44	48	18	41	27	44	38
	Cuprous oxide with bentonite (D) 0·25% Cu	—	20	71	11	25	20	52	32
	½% Bordeaux mixture (½B) 0·125% Cu	—	26	32	—	—	9	45	25
	½% Bordeaux with excess lime (½BX) 0·125% Cu	—	—	—	12	28	25	40	
2	Cuprous oxide with water-soluble dispersing agent (Perenox) (P) 0·25% Cu	14	13	24	12	21	9	17	18
	Copper oxychloride with water-soluble dispersing agent (Coppesan) (C) 0·25% Cu	9	7	16	8	16	—	—	12
	Metallic copper with bentonite (K) 0·25% Cu	—	14	17	8	27	—	—	17

In estimating the *percentage* retention of the spray deposits, over the several test periods, it became necessary to allow for the amount of copper in the unsprayed leaves, which was significant in relation to some of the meagre copper deposits left after weathering. The figures for copper in unsprayed leaves in 1942 are almost certainly high, and this may have been due to failure to take the obvious precaution (adopted in subsequent years) of collecting the unsprayed leaf samples first, so that the fingers were not contaminated with copper from handling sprayed foliage. Both in the later disk trials and in the washing trials described below, the copper in unsprayed leaves was found to be about 0·04 mg./50-disk sample, and this amount has been uniformly deducted from the mean values for *total* copper in working out the percentage retention figures given in Table 4.

The percentage figures in Table 4, when read with consideration of the standard errors of the means from which they are derived (Table 3), provide a convenient summary of the principal results obtained. The mean figures for all periods in 1942 and 1943 (in which all the treatments were included) are chosen as fairly indicative of the general performance of the sprays, as regards spray retention, in all the trials. It is considered that the limits of error in the sampling justify the placing of the treatments into two groups, the first with spray retention about twice that of the second, but with cuprous oxide and bentonite, and ½% Bordeaux mixtures, occupying an intermediate position.

Low-copper Fungicides

In the 1943 trial the treatments included three low-copper fungicides, applied twice only, at the same times as the sprays of normal copper dosage. The results are shown in Table 5.

Except with the ¼% Bordeaux mixture, the total amounts of copper found in the leaf samples taken at the ends of the trial periods were not significantly different from those in the unsprayed leaves; but the value of these low-copper fungicides intended primarily for horticultural use was not disclosed by our trials under farm conditions. Where more applications could be given (e.g. in our routine use of the low-copper material, Bouisol, at 0·125% Cu for the protection of special plots for virus disease and growth observations; vide Large (1945, Fig. 3) *et passim*), a sufficient amount of copper could be maintained on the leaves to keep the plants almost completely free from blight throughout their growing period. Fig. 2 shows the protective effect of six applications of ½% Bordeaux mixture, in relation to dates of spraying, rainfall, and amounts of copper deposit maintained on the leaves.

Battery Washing Method

In 1944 a battery washing method was tried in comparison with the disk method already described. For the washing method samples of twenty entire compound leaves were taken, five from each of the four

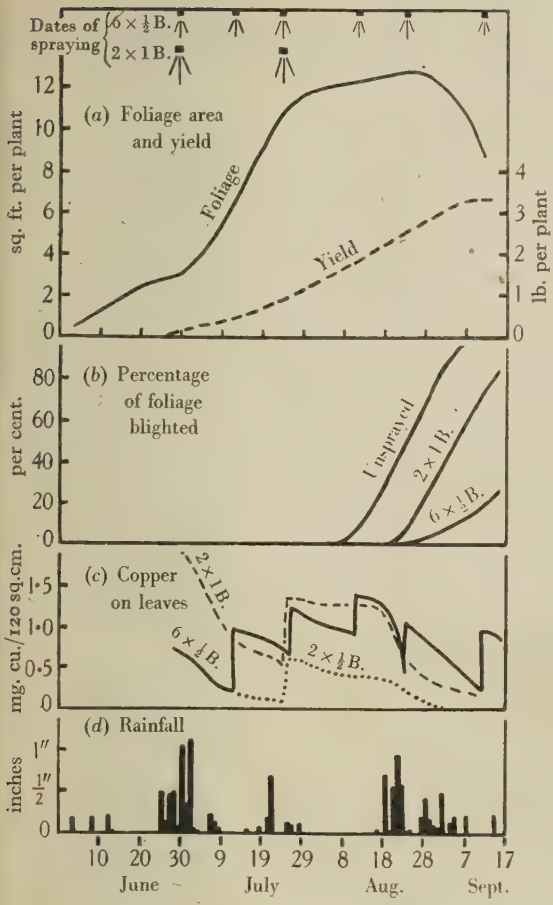

Fig. 2. Spray retention in relation to dates of spraying, expanse of foliage, yield, progress of potato blight attack, and rainfall, Majestic, 1944.

TABLE 5. *Preliminary trial of low-copper fungicides: mean total copper in leaf-samples*

(0·01 mg. Cu/120 sq.cm.)

	Rain 5·2 in.		Rain 1·9 in.	
	7 July	6 Aug.	7 Aug.	27 Sep.
¼% Bordeaux mixture with excess lime (¼BX) 0·07% Cu	45	6	86	30
Bouisol-white oil emulsion 10 pt./100 gal. (Bwo) 0·04% Cu	20	5	20	5
Fungex, cuprammonium compound 5 pt./100 gal. (F) 0·06% Cu	25	6	32	7
Unsprayed	5	4	5	4

plots for each treatment. These whole leaves were collected on the same dates as the disk samples and from the same parts of the plants. The leaves were taken into the laboratory immediately after collection, and the copper on them was removed for estimation by plunging them one at a time into seven beakers in turn, the first six beakers containing 500 ml. of $\frac{1}{2}N$ acetic acid, and the last 500 ml. of distilled water. Each leaf, held by the stalk, was submerged and agitated continuously for 1½ min. in each beaker before transference to the next. The twenty leaves were passed in turn through each of the seven beakers. The washings were then mixed, measured for volume, and the copper in them was estimated by the diethyldithiocarbamate method, using a Spekker absorptiometer, as previously described.

Where the leaves had been sprayed with cuprous oxide, a mixture of 1% nitric acid and 2% hydrochloric acid was substituted for the $\frac{1}{2}N$ acetic. The carbamate colour complex was then found to fade within 15 min., but if readings were made within 5 min. of the addition of the reagent the accuracy of the method was not affected.

The efficiency of the washing method was tested by determination of the amount of copper removed in each successive wash, and the amount of copper remaining in the leaves after washing. Typical test results with leaves taken shortly after spraying are given in Table 6.

The total copper remaining in the washed leaves, as determined by disking and wet ashing, was of the same order as that in unsprayed leaves, and with both acetic acid and aqua regia as solvents about 99% of the copper on the leaf surfaces was removed by the first three washings.

To determine the area of the leaves, each sample of twenty was blotted after washing, and a portion of unit area (120 sq.cm.) was quickly cut from the leaflets and weighed. The rest of the laminae were then stripped from the stalks between the fingers and also weighed. The total weight of the laminae divided by that of the unit sample, and multiplied by 120, was taken as the area of the sample in sq.cm. As the two weighings were made within a few minutes of each other, differences in specific weight due to differences in loss by evaporation from the two portions were negligible. This simple method of area determination was found to give results agreeing very closely with planimeter measurements of contact leaf prints made in a blue-print machine. The small error, almost equally affecting all samples, due to non-inclusion of the leaf stalks, was ignored.

From the total amount of copper recovered in the washings, and the area of the leaves as determined above, the equivalent copper deposits per 120 sq.cm. were calculated for comparison with the results obtained by the disk method, as shown in Table 7.

There was fairly good agreement between the

results by the washing and the disk method in the second trial period, but not in the first. The area of the twenty-leaf samples taken for the washing method was about 3000 sq.cm., or six times that of the four samples of fifty disks taken for the disk method. The whole-leaf samples were better representative than the disks of the distribution of spray deposits over entire leaves, but as they were taken from only twenty random sites among the four plots, they were less well representative of the distribution of spray over the whole expanse of the foliage than the disk samples from 200 random sites. With the washing procedure worked out, we should, in another year, cut the number of washings to four, and take at maximum, and there was not then very much new growth before the second sampling. Thus, throughout all the trials, the results in the second test period each year must be regarded as more reliable than those in the first period.

The results of the washing trials, however, again showed the good retention of Bordeaux mixture, and supported the place order of the several other sprays in Table 4. The mean amounts of copper per unit leaf area for all treatments, and at each of the four sampling dates, by the washing method, were respectively 139, 136, 122 and 131% of those by the disk method. This was consistent with the results of the preliminary trial of spray placement (Table 1),

TABLE 6. *Progress of copper-removal in battery washing*

(0·01 mg. Cu/120 sq.cm. leaf area)

	Removed in wash no.							Remaining in leaves after washing
	1	2	3	4	5	6	7	
Bordeaux mixture using $\frac{1}{2}N$ acetic	104·0	14·2	1·6	0·5	0·4	0·2	Trace	4·5
Cuprous oxide using 1% HNO_3+2% HCl	107·0	12·8	3·0	1·1	0·6	0·4	Trace	3·2

TABLE 7. *Estimates of spray retention by disk and by battery washing methods compared.*

D=disk method, W=washing method

(0·01 mg. Cu/120 sq.cm.)

Rain (in.) ...		3·9			3·2		
		29 June	24 July	%	25 July	23 Aug.	%
1% Bordeaux	W	217	107	49	137	75	55
	D	182	49	27	134	59	44
Cuprous oxide and bentonite (D)	W	162	15	9	175	95	54
	D	113	22	20	138	72	52
$\frac{1}{2}$% Bordeaux	W	91	16	18	68	31	46
	D	68	6	9	55	25	45
$\frac{1}{2}$% Bordeaux with excess lime	W	104	10	10	83	39	47
	D	48	12	25	50	20	40
Perenox	W	140	16	11	130	18	14
	D	101	9	9	88	15	17
Potash Burgundy	W	—	102	—	158	70	44
	D	—	97	—	150	58	39

least three independent samples of twenty leaves for each treatment. This would reduce the sampling error, but the true cause of the greater irregularity of the results in the first period than in the second is undoubtedly to be seen in Fig. 2*a*. On the date of spraying, at the beginning of the first period, the expanse of foliage was only about a quarter of that at the end of the period. Despite every care to take the end samples from the parts of the plants which had received the spray, and not from the new growth, it was, in fact, impossible to avoid including some unsprayed leaves, and the striking irregularities of the results for the first trial period in Table 7 usefully emphasize this fact. At the time of the second spraying the expanse of foliage was approaching its and could be accounted for by the higher concentration of the spray deposits at the leaf tips, which were included in the washing samples but not in the disks.

ABSORPTION OF COPPER BY THE LEAVES

(1) *Greenhouse trial*

The apparent completeness with which superficial deposits of copper could be removed by the method of battery washing encouraged us in an attempt to determine whether or not copper is absorbed into the potato leaves from the spray deposits—a riddle now 60 years old. A preliminary trial was made on greenhouse plants (var. Ulster Monarch). Three

blocks, each of eight uniformly well-grown plants, were treated as follows:

(1) Evenly sprayed with Bordeaux mixture on 18 Apr.
(2) Evenly sprayed with Bordeaux mixture on 10 May.
(3) Unsprayed.

Over the test period of 22 days, from 18 Apr. to 10 May, the foliage of the plants in block 1 had an opportunity to absorb copper from spray deposits, which was not shared by those in block 2. Some simulation of the natural weathering of the deposits in the field was assisted by watering the foliage twice daily with a fine rose. On 10 May, 2 hr. after the spray deposits in block 2 had dried, two entire sample leaves were taken from each plant in all three of the blocks, those in block 1 having been marked at the time of spraying to ensure that new growth was not included.

The samples of sixteen whole leaves from each block were then washed by the battery method, until the trace of copper in the last wash, if detectable at all, corresponded to less than 0·002 mg. Cu/120 sq.cm.

Table 8. *Total copper in potato leaves after exhaustive removal of surface deposits by battery washing: greenhouse trial*

(0·01 mg. Cu/120 sq.cm.)

22 days after spraying with Bordeaux mixture	4·5, 4·4, 4·0
4 hr. after spraying with Bordeaux mixture	2·5, 2·3, 2·7
Unsprayed	2·5, 2·7, 2·4

Three samples of fifty standard disks were cut from each batch of sixteen washed leaves, and the total copper in the disks, after wet ashing, was determined by the usual method, but with 4 cm. instead of 1 cm. cells in the Spekker, which rendered it just possible to detect differences of the order of 0·00025 mg. Cu/50 ml., or 0·001 mg. Cu/50-disk sample. The results are shown in Table 8.

Thus the leaves which had been treated with Bordeaux mixture, but which had only 4 hr. in which to absorb copper from the spray deposits, were found to contain no more copper than the unsprayed, while those which had 22 days in which to absorb copper contained a significantly greater amount. There was thus a positive indication that about 0·02 mg. Cu/120 sq.cm. was either absorbed by the leaves, or so tenaciously adsorbed on the leaf surface, perhaps as a result of weathering, that it resisted removal by six washings with $\frac{1}{2}N$ acetic acid.

(2) *Field trial*

Three samples of fifty standard disks were cut from each batch of twenty washed leaves, left after the determination of copper deposits by the battery washing method, for the samplings on 24 July and 23 Aug. in the 1944 field trial of spray retention. These disk samples from the washed leaves were then independently ashed and analysed for copper, with the results shown in Table 9.

The leaves taken on 24 July had opportunity to absorb copper from spray deposits for a period of 25 days; and those taken on 23 Aug. for a further 30 days. Again there was an indication of slightly more copper in or on the washed leaves that had been sprayed with Bordeaux mixture than in the unsprayed.

Measurement of 'leaf deckage' and the expanse of foliage in a potato field

The 'unit and total weight' method of determining foliage area, used in the washing method described above, was applied in the 1944 trial to the estimation of the total expanse of potato foliage per acre on successive dates. As there was plenty of material,

Table 9. *Total copper in potato leaves after exhaustive removal of surface deposits by battery washing: field trial. Means of three estimates*

(0·01 mg. Cu/120 sq.cm.)

	24 July	23 Aug.
1% Bordeaux mixture	5·3	4·9
Cuprous oxide and bentonite	4·5	4·6
½% Bordeaux mixture	2·7	2·4
½% Bordeaux mixture with excess lime	1·8	3·2
Perenox	1·8	3·0
Unsprayed	1·5	2·4
Significant difference (P=0·05)	3·0	1·0

and no objection to the requisite sampling, the method was preferred to the logarithmic leaf-grading procedure of Bald (1943), and to other methods involving itemization of visual assessments. Typical samples of ten consecutive plants, in the plots which it was hoped to keep protected from blight over the whole growing period by six applications of ½% Bordeaux mixture, were lifted on each date. The growth in all the other plots was much the same apart from blight injury after the middle of August. All the leaflets on the sample plants were stripped into a basket and weighed on the field. A number of fifty-disk samples were then cut from the leaflets and placed in tared tubes for weighing in the laboratory. The specific weight of the foliage per 120 sq.cm. varied from 3 to 4 g. according to the stage of growth and weather conditions at sampling.

Fig. 2*a* shows the mean area of the foliage in sq.ft. per plant from the beginning of June to mid-September, and also the corresponding crop estimate, obtained by weighing the tubers on the sample plants. The mean spacing of the plants, determined with considerable accuracy when lifting at the end of

the season, was 22 × 27 in., and the mean plant population was 10,600/acre.

At the time of the first spraying, on 29 June, the expanse of foliage was about ¾ acre/acre. Through July the haulm was growing very rapidly (following the rain, as shown in Fig. 2*d*), and at the time of the second spraying, 25 July, the expanse of foliage was about 3 acres/acre. Thus, at the end of the first trial period, three-quarters of the foliage was unsprayed, and apart from a slight possible secondary distribution of the initial spray deposits by drip, seepage, or splashing, was entirely unprotected.

Practical considerations

Whatever the tenacity of the spray material, the first spraying cannot be expected to give adequate protection for 3–4 weeks when the foliage is rapidly expanding, and in such circumstances what is really needed is an intermediate spraying, as given to our 'continuously protected' plots in 1944 (Fig. 2*a*). In this particular trial, however, the blight attack did not in fact begin until the first week in August (Fig. 2*b*), and as there was only a relatively small increase in the leaf deckage after 24 July, the second spraying on that date was capable of protecting the foliage for a period depending chiefly on the tenacity of the spray deposits. Fig. 2*c*, *d* provides an approximate history of the amount of copper on the sprayed leaves in relation to the rainfall. It is in the endeavour to obtain as good as possible a control of blight with two sprayings (or even with a single late spraying when the risk of an early attack is slight) that high tenacity in the spray material is of the greatest importance.

All the present trials were, in effect, studies of a farming operation, a practical compromise, by which the control of potato blight is attempted with one precautionary spraying on rapidly expanding foliage, and one main spraying, which can be much more fully effective, at or near the point of maximum leaf deckage.

It would be very desirable to know what minimum coverage of copper must be maintained on potato leaves for their effective protection against potato blight. Our trials, with two sprayings only, were not designed to provide information on this point, but it was found that repeated applications of ½% Bordeaux mixture, maintaining the spray deposits between 0·5 and 1·0 mg. Cu/120 sq.cm., was sufficient to give full protection until very late in the season. With maximum leaf deckage at, say, 4 acres/acre, this was equivalent to 1½–3 lb. Cu/acre of crop in full growth.

In our trials the mechanical efficiency of the spraying process was fairly high. Tables 3 and 7 show that for about 4 lb. of copper distributed at the second spraying (160 gal./acre 0·25% Cu) from 1·0 to 1·5 mg. Cu/120 sq.cm. was deposited on the leaves. With leaf deckage about 3 acres/acre at the date of application, this was equivalent to 2¼–3¼ lb. of copper reaching and remaining on the leaves.

Conclusions

With leaf samplings made in such a way as to test the variations in coverage of spray deposits that must occur over any expanse of sprayed foliage, it was found that the disk method of spray retention estimation, with four to six independent samples per treatment, gave results which could be regarded as reliable within ± 10%.

About 99% of the total copper on the leaves could be removed in three stages of the alternative battery-washing method described, and results obtained by this method were about 30% higher than those by the disk method, owing to inclusion of the denser copper deposits at the leaf tips.

The percentage retention of 1% Bordeaux mixture over periods of 3–4 weeks with 2½–3½ in. of rain was about 40%. That of cuprous oxide and copper oxychloride sprays compounded with sulphite lye or other water-soluble dispersing agent was less than 20%. Compounding of cuprous oxide with bentonite as an insoluble sticker and dispersing agent gave improved adhesion, but the results were irregular. In six trials out of seven the percentage retention of ½% Bordeaux mixture, with or without excess lime, was significantly lower than that of the standard 1% mixture.

A general indication from all the trials was that maintenance of spray deposits at not less than 0·5 mg. Cu/120 sq.cm. over the whole expanse of foliage would give efficient protection against potato blight, under moderately severe conditions of incidence; and that *provided sufficient applications were given*, according to the copper dosage and/or tenacity of the compounded spray material, to maintain the required amount of copper on the leaves, equally good protection could be obtained with any of the copper fungicides included in the trials (Bordeaux mixture, Burgundy mixture, cuprous oxide, copper oxychloride, or even finely divided metallic copper).

A slight absorption, or acid-resistant adsorption, of copper from spray deposits by potato leaves, of the order of 0·02 mg. Cu/120 sq.cm. of leaf area, was indicated but not proven.

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The cereals root eelworm, *Heterodera major* (O. Schmidt) Franklin, in North Wales

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(With Plate 5 and 1 Text-figure)

The cereals root eelworm *Heterodera major* (O. Schmidt) is shown to be locally distributed in North Wales, the most heavily infested centres being in the arable districts of the eastern counties of the province. The most severe attacks occurred on oats where the crop had been grown too frequently; wheat and barley were apparently unaffected at the cyst populations encountered.

INTRODUCTION

Franklin (1940) has shown that the oat strain of *Heterodera schachtii* Schmidt is a distinct species and should be designated *H. major* (O. Schmidt, 1930). A brief historical account of this eelworm and an account of the losses caused in the west midlands have been given by Edwards (1935). The object of the present note is to record the position in North Wales and in particular to give details of individual fields (identified by their Ordnance Survey numbers) as a preliminary to a more intensive study.

The cyst counts recorded are mainly the number of cysts per 100 g. of air-dried soil but, for more detailed comparisons, annual counts of viable cysts and eggs are now being made for a number of fields. The lactophenol technique (Goodey, 1937) was employed for examination of eelworms *in situ* in plant tissue.

DISTRIBUTION IN NORTH WALES

After the discovery of the first affected field in North Wales in 1943 a fairly intensive search showed some forty-two fields, situated on ten farms, to be infested; cyst counts varied from 1 to 66 per 100 g. soil. Practically all the infested fields were found to have a history of rather intensive cereal growing and are situated in arable districts.

The most heavily infested district is an easily cultivated light land area in east Flintshire where it is difficult to establish a good grass sward (Text-fig. 1, Area A). Area B is one farm where arable crops have been grown intensively for some 30 years. Area C consists of two fields, located as a result of describing eelworm symptoms at a farm demonstration. Area D is one field in which the fourth oat crop after being seeded down for 7 years was a complete failure. One corner of a field at Aber near Bangor

Text-fig. 1. Showing distribution of *Heterodera major* (O. Schmidt) Franklin. × Situation of infested farms.

has also been shown to be lightly infested (not shown on map).

Relation between degree of infestation and previous cropping

Losses recorded have ranged from very light infestations, discovered only as a result of careful examination of cereal roots, to total failures. Pl. 5, fig. 1 shows the appearance of patches in a field at about the stage when a farmer first realizes that something is wrong either with the soil or with the plant. Pl. 5, fig. 2 shows a mixed crop of oats, barley and peas, in which the oats alone suffered severely.

The crop rotations and cyst counts of a number of infested fields are shown in Tables 1 and 2.

Although *H. major* has been reported from widely separated districts, the distribution of this eelworm is not, at the moment, very well known. It has been shown that it is only locally distributed in North Wales. Reference to the cropping records given in Tables 1 and 2 shows that complete failures generally occurred only after too frequent cropping with oats. Excellent crops of barley and wheat were grown on heavily infested land and, except for one crop of winter wheat, few cysts were present on the roots of these crops. In addition to the cereal crops, cysts were found on the roots of perennial rye-grass

Table 1. *The cropping of eelworm-infested fields in Area B, Ordnance Survey Map No. XL N.W.*

B = barley, G = grass, O = oats, R = roots, Ra = rape, S = seeds, W = wheat

Field no. ...	201	203	526	641	646	647	649	514
1924	R	S	R	O	O	W	W	
1925	O	G	O	W	S	R	O	
1926	S	G	O	O	G	O	R	
1927	G	G	S	R & O	W	S	O	
1928	W	W	G	W & O	O	G	S	
1929	O	B	W	S	R	W	G	
1930	R	R	O	G	O	O	W	
1931	O	O	R	W	S	R	O	
1932	S	S	W	O	O	O	R	
1933	O	O	S	R	W	S	O	
1934	W	W	O	O	R	O	S	
1935	R	R	W	S	O	W	O	
1936	O	O	R	O	S	R	W	
1937	S	S	O	W	O	O	R	O & R
1938	O	O	S	R	W	S	O	O & R
1939	W	W	O	O	R	O	S	O & S
1940	R	R	W	S	O	W	O	S
1941	O	O	R	O	S	R	W	O
1942	S	S	O	W	O	O	R	O
1943	O	O*	S	R	W	S	O	O†
1944	W	W	G	O*	R	O*	S	Ra
Cysts/ 100 g.	13	27	20	52	24	42	31	45

* Poor crop. † Failure.

Fig. 1

Fig. 2

TABLE 2. *The cropping of eelworm-infested fields in Areas A, C and D*

	Area A	Area A	Area A	Area A	Area C	Area D
O.S. Map No. ...	XXIII S.E.	XXIII S.E.	XXVI N.W.	XXIII S.W.	XIX S.E.	XXXV S.W.
Field no. ...	497	487	164	241 and 243	738	612
1933	—	—	—	—	Seeds	Seeds
1934	—	—	—	—	Grass	Grass
1935	—	—	—	—	Grass	Grass
1936	—	—	—	—	Oats and seeds	Grass
1937	—	—	—	—	Oats and grass	Grass
1938	—	—	—	—	Oats and grass	Grass
1939	—	—	—	—	Seeds and grass	Grass
1940	Seeds	—	Oats	Oats	Grass and oats	Oats
1941	Grass and roots	Seeds	Roots	Oats	Oats	Oats
1942	Oats	Oats	Clover	Oats	Oats	Oats* and roots
1943	Oats and roots	Barley	Oats and peas	Oats	Oats†	Seeds and oats†
1944	Oats*	Oats†	Oats† and peas	Oats*, potatoes and wheat	Rape	Seeds
Cysts/100 g.	66	51	27	45	35	52

* Poor crop. † Failure.

(*Lolium perenne*), sterile brome (*Bromus sterilis*) and *Avena fatua*. None of the various other weeds examined for the presence of larvae in the roots and cysts on the roots was found to be attacked. Cysts, probably not those of *Heterodera major*, were also found on roots of red clover (*Trifolium pratense*).

The writers desire to express their gratitude to the owners of the fields examined for allowing them complete freedom to examine the crops at all times and for supplying details of rotations over a long period. They also thank Mr A. W. Colling, B.Sc., for the photograph which forms Pl. 5, fig. 2.

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EXPLANATION OF PLATE 5

Fig. 1. Early symptoms of the cereals root eelworm *Heterodera major* (O. Schmidt) Franklin.

Fig. 2. Mixed crop of barley, oats and peas in which the oats has been attacked by the cereals root eelworm *Heterodera major* (O. Schmidt) Franklin.

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The shallot aphis, *Myzus ascalonicus* Doncaster, and its behaviour as a vector of plant viruses

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(With Plate 6)

A new species of aphis, *Myzus ascalonicus* Doncaster, is briefly described, and compared with *Myzus persicae* Sulz., which it resembles superficially. It has been found on shallots in storage and on onions and other species of plants both in glasshouses and in the open between October and June. Its summer habits and hosts are unknown. In comparative virus transmission tests with *M. persicae* it was found that *Myzus ascalonicus* transmits dandelion yellow mosaic virus, which is not transmitted by *Myzus persicae*; and also cucumber virus I, *Hyoscyamus* virus III and sugar-beet yellows virus, all of which are transmitted by *M. persicae*. *Myzus ascalonicus* does not transmit the viruses of potato *Y*, severe etch, lettuce mosaic and sugar-beet mosaic which are transmitted by *Myzus persicae*.

Introduction

Since 1941 what appears to be a new species of aphis has been found in increasing numbers on a variety of host plants. It occurs most commonly on shallots and allied plants, both on the bulbs in storage and on the growing plants. This aphis has been named *Myzus ascalonicus* Doncaster, and its anatomy and morphology described in detail elsewhere (Doncaster, 1946). Superficially it resembles *Myzus persicae* Sulz. but differs from the latter both morphologically and biologically. It is an efficient vector of several viruses, some of which are not transmissible by *M. persicae*, and, as it appears to have a wide host range, it is clearly a potential danger not only as a pest of onions but as a transmitter of virus diseases.

Brief description (Plate 6)

1. *Apterous viviparous female.* Body uniformly pale brown, greenish brown, or dull yellow in all stages; occasionally some adults show irregular dark olive green markings on the abdomen; dorsum strongly convex, shining. Head with prominent frontal tubercles, projecting inwards and more pronounced than those of *M. persicae*. Eyes black. Antennae as long as, or longer than the body, apex of joint V and the whole of VI dark. Legs pale, the tarsi and apices of the tibiae dark. Cornicles pale, slender, rather short, distinctly swollen on apical half. Cauda small, pale, bluntly triangular. The cornicles are proportionately smaller in relation to the rest of the insect than those of *M. persicae*. A useful criterion for comparison between the two species is the proportion of cornicle to antennal joint III: in *M. ascalonicus* this is about 1 : $1\frac{2}{3}$; in *M. persicae* about 1 : $\frac{4}{5}$.

2. *Alate viviparous female.* Similar in general shape and colour to *M. persicae*. Head, thorax and abdominal markings deep black. The numbers of rhinaria on the antennae are extremely variable, and may range from a few near the base of the third joint to large numbers covering the whole of joints III, IV and V. Frontal tubercles much as in aptera. Antennae slightly longer than the body, black. Legs, except for bases of femora, black. Cornicles and cauda black, otherwise as in aptera. Proportion of cornicle to antennal joint III: *M. ascalonicus*: 1 : $2\frac{2}{5}$; *M. persicae*: 1 : $1\frac{1}{3}$.

Occurrence and distribution

As yet *M. ascalonicus* has been found only during the winter and spring, and nothing is known of its habits during the period June–October. It was first encountered in December 1941 causing damage to stored shallots in Spalding, and since then it has been found frequently on both onions and shallots in storage in Lincolnshire and Hertfordshire during the winter. The aphides feed on the scale leaves under the outer skin, and on the young shoots, and are often present in sufficient numbers to render the bulbs unfit for planting. Onions of various kinds appear to be the most favoured hosts, but the aphis will feed and reproduce readily on a variety of other plants. Glasshouses seem to provide conditions particularly suited to its development, and infestations, sometimes reaching serious proportions, have been found on winter lettuce in Bedfordshire and Lincolnshire during February and March. On one occasion (14 Apr. 1943) alatae were observed migrating from infested lettuces to onion seedlings in a glasshouse in Spalding. And on 28 Mar. 1944, a very heavy infestation was found on a bed of cabbage seedlings in a heated glasshouse in Lincolnshire. The aphides were spreading from the seedlings to weeds (*Stellaria media* Vill. etc.). Other host plants on which *M.*

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ascalonicus has been found under glass include *Polygonum persicaria* L. and *Taraxacum officinale* Weber.

No sexual forms have been found, and the evidence so far available indicates that the aphis normally passes the winter as apterae viviparae in stores and glasshouses. Occasional instances of its occurrence on outdoor plants in the early spring suggest that it may also overwinter in sheltered situations in the open. In April 1944 and again in March 1945 small colonies of apterae were found on plants of *Aubrietia* in a private garden in Harpenden, and in February 1945 a single adult aptera and two nymphs were found on savoy cabbage in Spalding. Other host plants on which the aphis has been found in the open include *Kentranthus ruber* DC. (12 May 1942), *Arabis* sp. (garden variety, 15 Apr. 1943), *Geranium sanguineum* L. (8 May 1944), *Campanula* sp. (Apr. 1945) and *Crepis biennis* L. (11 June 1945).

TABLE 1

Locality	*M. ascalonicus* Alatae per sq.ft. trap surface 1942	1943	1944
Rothamsted Farm	2·7	10·8	8·4
Rothamsted Allotments	—	—	6·2
Writtle, Essex	—	0·5	—
Woburn, Beds	0	2·7	3·0
Lincoln	—	—	0·3
Welton Heath, Lincs	—	1·9	—
Spalding, Lincs	0·8	3·5	1·9
Spalding Marsh, Lincs	—	0·8	—
Baston Fen, Lincs	—	2·2	1·9
Bourne Fen, Lincs	0·3	—	—
Charnwood, Notts	—	0·3	—
Newport, Shropshire	—	1·1	0·5
Newton Abbot, Devon	—	0·5	1·9
Truro, Cornwall	—	—	0·3
Edinburgh	—	4·6	6·2
Dundee	—	—	1·1

In 1942, 1943 and 1944 a number of simple adhesive insect traps were employed to gain information on aphis migration in different parts of Britain. The traps consisted of metal tubes, 3 ft. long and 5 in. diam., painted white and mounted vertically with their tops about 6 ft. from the ground. The surface was coated with grease-banding material. Aphides of all species were removed and preserved for identification at weekly or fortnightly intervals, and the trap cleaned and recoated with grease. The willing co-operation of advisory entomologists and others made possible the collection of data on aphis migration from localities all over Britain, and a full report of this work is in preparation. Alatae of *M. ascalonicus* were taken in nearly all the localities where traps were operated. In each of the 3 years 1942–44 alatae occurred on traps during May and June, and again during the latter half of October and early November. The localities of the traps and the total numbers of alate *M. ascalonicus* taken in each locality are set out in Table 1. At many centres trappings were discontinued in September or October; had the records at these centres been continued to include the late autumn migration of this aphis, the numbers trapped would probably have been higher.

VIRUS TRANSMISSION TESTS

Transmission experiments were carried out with eight viruses. With each virus quantitative tests were made to compare the relative efficiency of *M. persicae* and *M. ascalonicus* as vectors. Six of these viruses belong to the group called 'non-persistent' by Watson & Roberts (1939). Vectors of this group transmit optimally if they are given a preliminary period of fasting, followed by a short infective feeding. To obtain the maximum degree of infection, and to standardize conditions, the aphides were starved for 4 hr. before they were fed for from 2 to 5 min. on leaves infected with these six viruses. With dandelion yellow mosaic (Kassanis, 1944) and sugar-beet yellows (Watson, 1940) the efficiency of the vector is increased with increasing feeding time on the infected plant. With these two viruses, the test insects were fed for one day on sources of the viruses. In all experiments the aphides were taken from these sources and immediately transferred to the healthy test plants, which were fumigated 24 hr. later.

As test plants, tobacco was used for potato virus *Y*, cucumber virus I, *Hyoscyamus* virus III and severe etch virus; lettuce for lettuce mosaic and dandelion yellow mosaic viruses, and sugar beet for the two sugar-beet viruses.

For the purposes of the virus transmission experiments, cultures of *M. ascalonicus* were maintained in the glasshouse on potted shallots, on which plant the aphis will feed and reproduce throughout the year. The results are recorded in Table 2.

Both aphides transmit cucumber virus I to the same extent. *Hyoscyamus* virus III and sugar-beet yellows are also transmitted by both aphides, but *M. ascalonicus* is the less efficient vector. *M. ascalonicus* will transmit dandelion yellow mosaic, which is not transmitted by *M. persicae*, but it will not transmit potato virus *Y*, severe etch, lettuce mosaic and sugar-beet mosaic, which are all transmitted by *M. persicae*.

One other interesting fact is that *M. ascalonicus* is the only known vector which will transmit cucumber virus I and *Hyoscyamus* virus III, but not potato virus *Y* and severe etch virus. Using this aphis, it has been possible to separate a pure culture of cucumber virus I from a plant infected with a mixture of this and potato virus *Y*. Similarly, when fed on leaves containing *Hyoscyamus* virus III and severe etch virus, it transmitted only *Hyoscyamus* virus III.

TABLE 2. *Comparative transmission of different viruses by* Myzus persicae *and* Myzus ascalonicus

Aphis	Virus	Aphides per plant	Treatment of aphides: Starving time before infective feeding	Treatment of aphides: Infective feeding time	Infected plants / Tested plants
M. persicae	Potato	8	4 hr.	2–5 min.	19/20
M. ascalonicus	virus *Y*	15	,,	,,	0/47
M. persicae	Cucumber	13	,,	,,	18/20
M. ascalonicus	virus I	13	,,	,,	41/50
M. persicae	*Hyoscyamus*	10	,,	,,	10/10
M. ascalonicus	virus III	20	,,	,,	20/35
M. persicae	Severe etch	10	,,	,,	10/10
M. ascalonicus		20	,,	,,	0/30
M. persicae	Lettuce mosaic	13	,,	,,	24/24
M. ascalonicus		25	,,	,,	0/38
M. persicae	Sugar-beet	20	,,	,,	5/5
M. ascalonicus	mosaic	10	,,	,,	0/11
M. persicae	Dandelion	20	0 hr.	1 day	0/20
M. ascalonicus	yellow mosaic	15	,,	,,	7/33
M. persicae	Sugar-beet	20	,,	,,	5/5
M. ascalonicus	yellows	10	,,	,,	5/11

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EXPLANATION OF PLATE 6

Fig. 1. *Myzus ascalonicus* Doncaster. Apterous viviparous female.

Fig. 2. *Myzus persicae* Sulz. Apterous viviparous female.

(*Received* 17 *August* 1945)

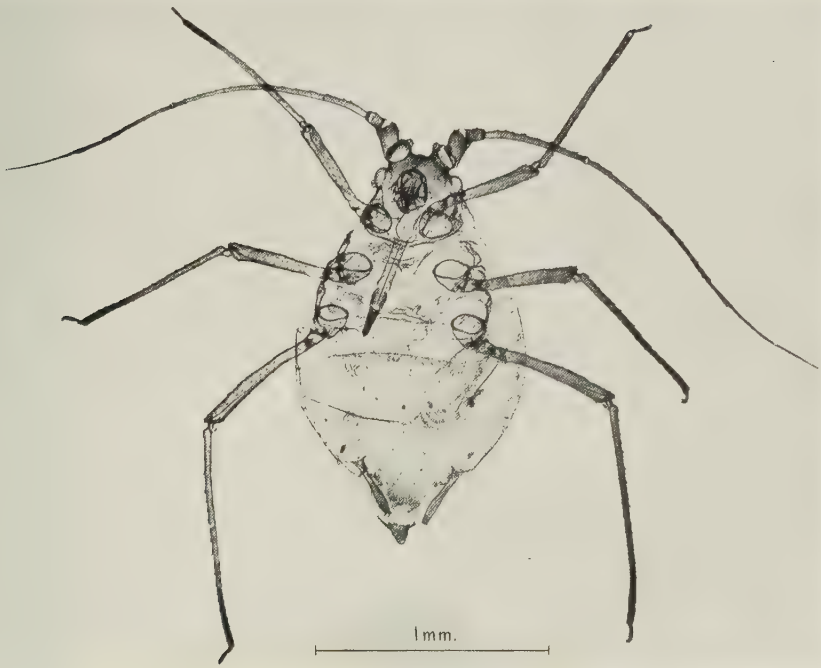

Fig. 1

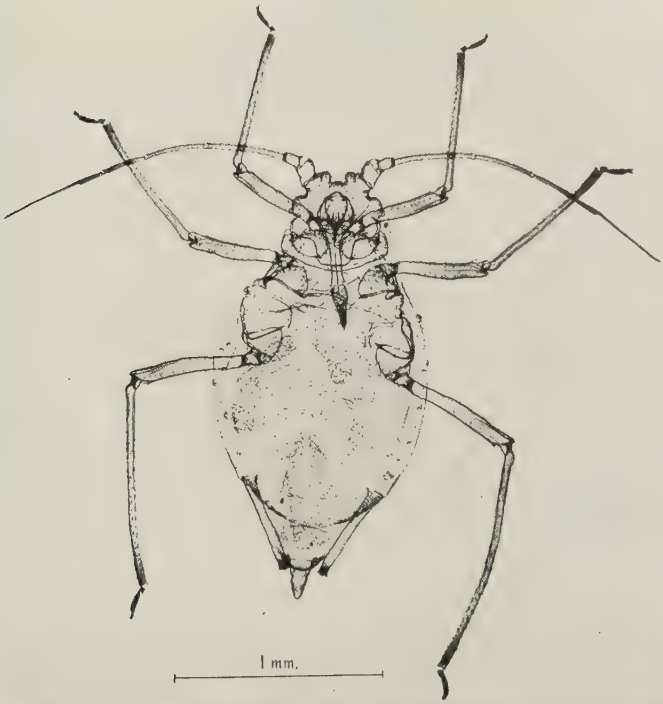

Fig. 2

Bionomics of the carrot fly (*Psila rosae* F.)

I. The infestation and sampling of carrot crops

By D. W. Wright, M.A., *Horticultural Research Station, Cambridge* and D. G. Ashby, B.A., *School of Agriculture, Cambridge*

(With 8 Text-figures)

The headlands of a carrot field are generally more heavily attacked by carrot fly larvae than the remainder of the field.

The methods of sampling a carrot crop are outlined: it has been found that, for fields up to 10 acres, samples of carrots taken in alternate twos and threes in the midfield, and in twos on the headlands, give a reliable measure of the infestation. For larger fields the number of carrots is doubled. Transects of headlands and whole fields have been made, and are useful in showing the actual amount of damage in a crop. Thus it is possible to represent each field as a three-dimensional body showing the amount and distribution of the attack.

The deterioration of attacked carrot crops during autumn and winter has been followed. Deterioration is measured by (1) percentage carrots attacked, (2) number of mines per 100 carrots and (3) the percentage of carrots unsaleable or unfit for market. The relations between (1) mines per 100 carrots and time, (2) percentage attack and time, (3) mines per 100 carrots and percentage attack, and (4) mines per 100 carrots and percentage unsaleability are stated. Based on the above relationships, methods of prediction of deterioration have been worked out.

The importance of shelter in determining the degree and position of attack is discussed and the relative effects of different types of shelter on infestation are compared.

Introduction

It is well known among growers in carrot-fly infested areas that the headlands of carrot crops are, as a rule, far more heavily attacked than the remainder of the field; furthermore, all plants are not attacked equally. This is readily confirmed by inspection of the crop in summer or autumn, when dead or severely damaged plants may be seen in the affected areas. The attack, therefore, may be said to vary both from place to place in the field and from root to root in the damaged areas.

Sampling

To allow for this difference of attack between the headlands and midfield, samples have been taken in two positions, one around the perimeter of the field, 1 yd. from the edge of the crop (the *headlands* sample), and the other along lines 30 yd. from the edge (the *midfield* sample). Neither sample gives a true measure of the *average* attack in the field, the midfield sample tending in small fields to underestimate the attack, and to overestimate it in large fields. The headland samples, being consistently worse than those from midfield, give in practically all cases a value far above the average for the field. Samples taken in these two positions in different fields do however enable the infestations on a 30-yd. wide strip around each field to be compared. Also by periodically sampling the same field the growth of the attack in these two positions can be followed.

To attempt to secure a random sample of roots, small groups of carrots were taken from a number of evenly spaced points in the position selected. To minimize the factor of choice a stick was thrown forward and the group of carrots nearest one end (marked) taken. This was repeated along each side of the field, the proportion of the total sample taken from a side being related to the length of that side. For fields up to 10 acres fifty carrots were taken from both the headland and midfield positions; for larger fields this number was doubled.

The number of carrots taken in each group was found greatly to influence the randomness of the sample and, from this, the estimation of the attack. In one field a series of samples was taken on 28 Sept. 1943 along a line 15 yd. from the edge of the field. The sampling unit varied from 1 (fifty carrots taken singly) to 50 (all taken at one point) and samples of 100 carrots were taken in groups of 10, 25 and 100. This sampling test was repeated in another field on 7 Dec. 1943 and the weights of the samples were then also recorded. The results are given in Table 1.

Table 1 shows that the extreme randomization of taking single carrots produced, in December, heavier samples in comparison with the other groups. This feature was also noted in the September samples. It

appears to be due to 'personal' choice in the field, since the marked end of the stick usually touches two or three carrots and the tendency is to take the largest. With maincrop carrots in early autumn, the largest roots are those which have not suffered attack and a sample taken singly tends then to have a smaller percentage attack than the true value. Further attack and migration of larvae gradually remove this difference by early winter. These features are shown in the table. The data also show that where

TABLE 1

No. in sample	Size of unit	Attack %	Average	Average weight of sample lb.	oz.
Samples taken 28 Sept. 1943					
50	1	12	10	—	—
50	1	8			
50	2 and 3*	18	17	—	—
50	2 and 3	16			
50	5	10	13	—	—
50	5	16			
50	50	6	11	—	—
50	50	16			
100	10	16	16	—	—
100	25	12	12	—	—
100	100	22	22	—	—
Samples taken 7 Dec. 1943					
50	1	30	24·5	9	11
50	1	20			
50	1	28			
50	1	20			
50	2	24	25·0	8	3
50	2	26			
50	2 and 3*	22	22·0	7	13·5
50	2 and 3	18			
50	2 and 3	30			
50	2 and 3	18			
50	3	30	30·0	—	—
50	3	30			
50	5	32	26·0	7	7
50	5	26			
50	5	30			
50	5	16			
50	10	8	15·0	7	1
50	10	22			
50	50	28	19·0	6	9
50	50	10			

* Two carrots and three carrots taken alternately.

the sampling unit was 10 or more very variable results were obtained. It would appear, therefore, that 5 is the maximum size of the group compatible with accuracy in samples of 50 and that smaller units are to be recommended. In this investigation all midfield samples were taken in alternate twos and threes, and on the headlands, where the sampling line is longer, in groups of two. In plot samples the unit of two was also used. Both fields and plots have been sampled at intervals throughout autumn and winter and the field results agree closely with those obtained from the plots, where the sampling error is much reduced.

Transects

While sampling at the two positions of headlands and midfield provides figures for attack and enables valid comparisons between fields to be made, it gives little information on the pattern of attack in the field. This has been investigated by sampling attacked fields along one headland and along lines parallel to that headland 5, 15, 30, 40 and 50 yd. out into the field. The data for three sets of samples are

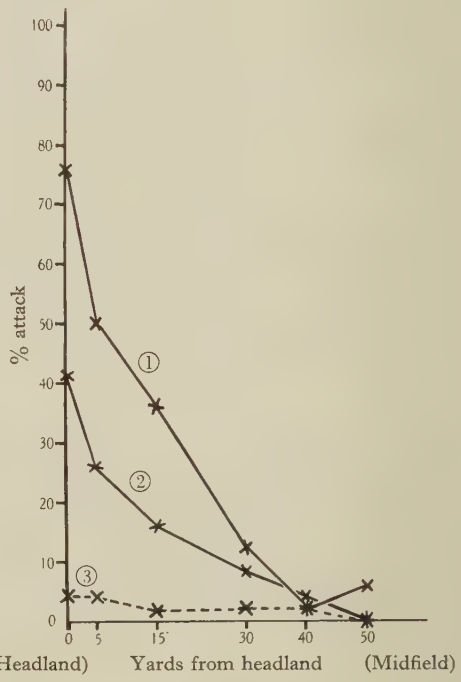

Fig. 1. Headland transects.

shown in Fig. 1, the curves produced being called *headland transects*. Each point on each curve is from a sample of fifty carrots taken in units of two. In each case the attack progressively decreases from headland towards midfield, a phenomenon called *headland effect*. Such curves are typical of fields showing slight infestations, the damage being chiefly located in a band around the perimeter of the field. Diagrammatic transects across a large and a small field are shown in Fig. 3. Both have incurred similar levels of headland infestation, but the proportion of the total crop damage is markedly different in the two cases. With high levels of attack, the middle of large fields may still be almost undamaged, but with

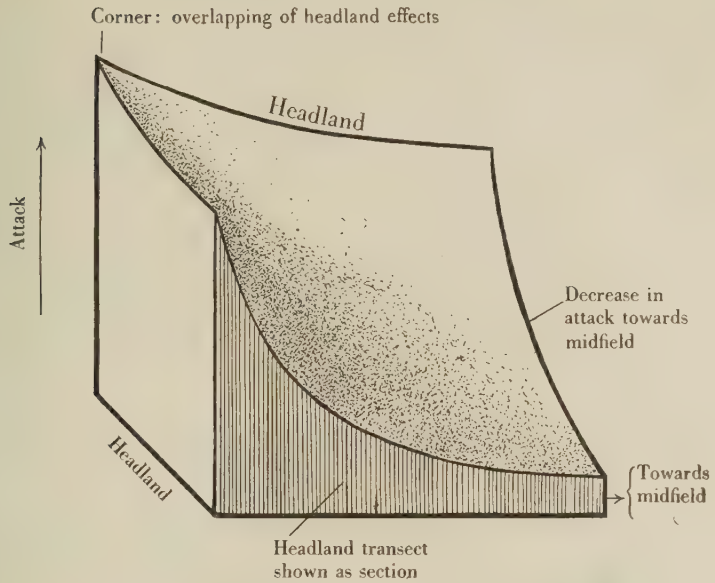

Fig. 2. Pictorial diagram of attack on the corner of a field.

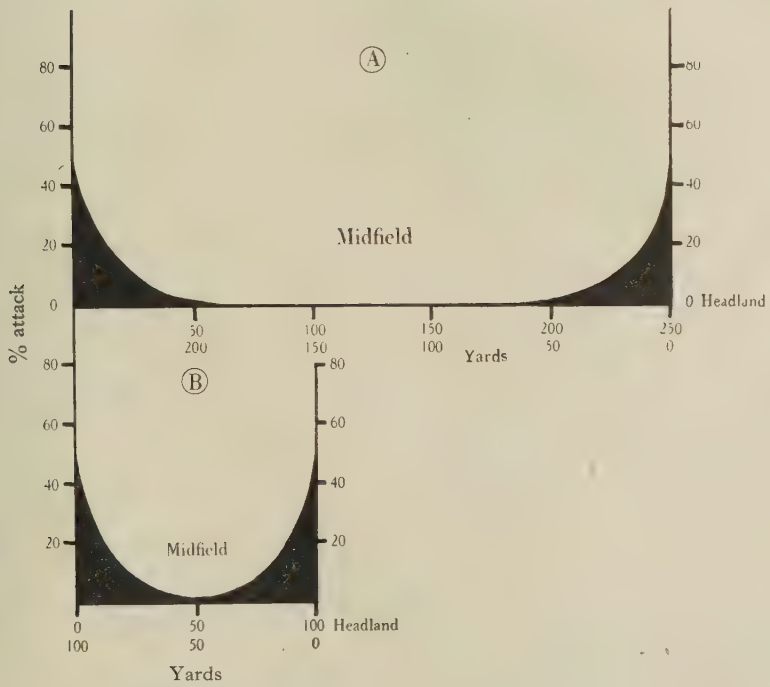

Fig. 3. Diagrams showing typical transects across a large field (A) and a small field (B). (Area under attack/distance curve is blacked in.)

small fields the attack will usually be high over the whole field and reach a maximum at the perimeter (see Fig. 4, curve 2). With very severe attacks, however, the damage then tends to be uniformly distributed over the whole field (see Fig. 4, curve 1).

Headland transects also show that different sides of a field and also adjoining sections along each side may suffer widely different attacks. The distribution of attack in the corner of a field is shown diagrammatically in Fig. 2, the headland attack being higher in the corner than elsewhere, apparently due to an overlapping at right angles of the headland effects. The cut edges of the solid show two different levels of headland attack and their decrease towards midfield. By making separate transects of the four headlands a three-dimensional figure can be constructed and the total percentage attack on the field estimated. The transects are made as far out into the field as the attack extends or until no further decrease in attack is observed.

Deterioration

The progressive increase in maggot damage through autumn and winter (deterioration) has been followed by the periodical sampling of thirteen fields and ten plots in 1941, 1942 and 1943. Three variables were recorded, the percentage attack, the percentage unsaleability and the number of mines per 100 carrots.

Unsaleability is the limit of damage which renders a carrot unfit for marketing; if one-third or more of the carrot has to be cut away to remove all damaged tissue, then it is classed 'unsaleable'. This agrees with the general standard used by the growers in sorting washed carrots for market. The *mine* is defined as the amount of carrot tissue damaged by the feeding of one larva during its development. Dissection has shown that this normally corresponds to an area about $\frac{3}{4}$ in. in diameter on the surface of the root and extending towards the core. Where mines pass around the root and into the core the number is assessed in relation to the area damaged and the number of maggots found. In heavily attacked roots where mines overlap and tunnels anastomose, errors in estimation probably occur.

Analysis of sampling results for both fields and plots has shown that the percentage attack, the percentage unsaleability and the number of mines per 100 carrots all increase with time, but not in the same relation.

Increase in number of mines with time

The increase in the number of mines per 100 roots during the period August to December is shown in Fig. 5. The data were obtained from two plots and from the headland and midfield positions of a field of maincrop carrots. Only damage caused by the second generation larvae was recorded. Although the slopes of the curves vary, an upward trend is definite in all.

Figures for mines per 100 carrots have been taken throughout the winter and spring in several cases, but as a general rule there is little increase in the number of mines after December, the bulk of the larval population having by this time passed the stage of rapid growth and feeding.

Increase in attack with time

The relation between percentage attack and time is shown in Fig. 6. The records were obtained from plots and from fields in the headland and midfield positions. They show the increase in attack at different levels of infestation during autumn and early winter.

Curve 1 is from a headland of a field having a heavy first generation attack during May and June. This attack is 'carried' through the whole year and the curve keeps consistently above the next lowest group. The field adjoining this was treated in May and June with sodium fluoride-molasses spray and the first generation attack largely prevented. The curve for this headland (2) is not separable from curves 3 and 4, given by maincrop carrots which received practically no first-generation attack. Curve 5 is of the headland and curve 6 of the midfield region of a field of maincrop carrots at Mepal, Isle of Ely, whilst curve 3 is of a plot on the same headland taken in the most heavily infested corner. Hence these three curves show the development of the same infestation at different levels of intensity and indicate that the rate of development of the second-generation attack throughout the autumn is approximately constant for all three levels. It follows that the most characteristic feature of the middle of a field is that it shows at any particular time a lighter and earlier phase of attack as compared with the headland. Although the individual curves do not fit well to a straight line, the similarity between curves 2, 3 and 4 shows that a simple straight-line relationship probably exists between the two variables. From the records shown in the figure and from other similar data it would appear that the slope of this line is approximately 30° from the horizontal for all levels of attack.

Relationship of mines per 100 carrots to percentage attack

The number of mines per 100 carrots is shown plotted against percentage attack in Fig. 7, the data being obtained from 104 samples. It will be seen that there is a close correlation between the two variables, such as would warrant the drawing of the curve shown in the figure. Such a curve is of considerable value since it enables the total damage (as mines per 100 carrots) in a sample to be determined by grading for attack only.

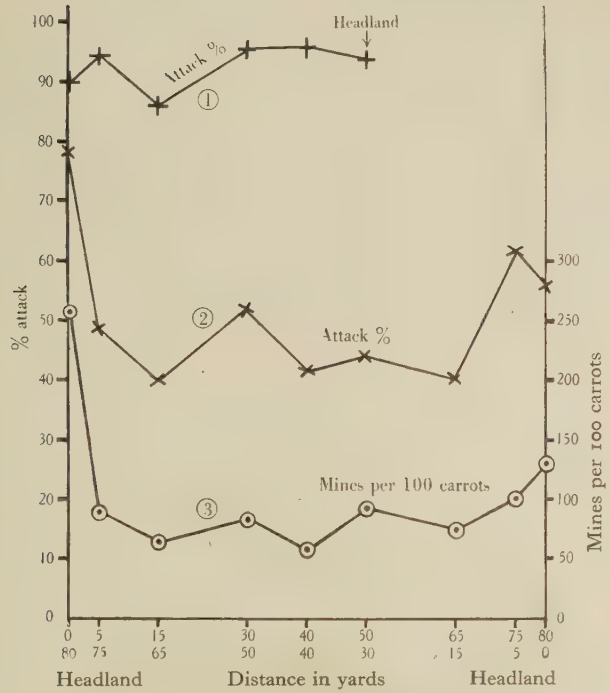

Fig. 4. Field transects.

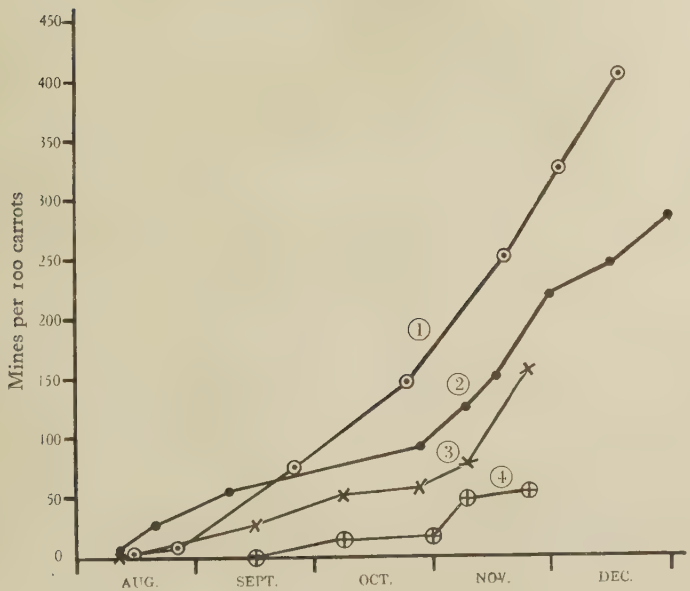

Fig. 5. Increase of mines per 100 carrots with time.

① Plot at Cambridge.
② Plot at Mepal, Isle of Ely.
③ Headland of field at Mepal.
④ Midfield of same

Relationship between mines and unsaleability

Using as a standard the fact that if one-third of the root is damaged the carrot is unsaleable it follows that the number of mines necessary to produce this will vary according to the size of the root. For large roots the number of mines required to give unsaleability is from 9 to 15, for medium-sized roots from 5 to 8 mines and for small roots from 1 to 4 mines. The corresponding root weights for these classes are over 8, 4–8, and less than 4 oz. respectively. Thus the relationship between mines per 100 carrots and percentage unsaleability will vary with the average size of the root in the sample. Fig. 8, curve 1 calculated regressions and may be used for reading off mines and unsaleability against each other.

Prediction

The relationships between attack, mines and unsaleability outlined above, makes possible certain predictions on the state of an attacked crop over a limited period from autumn to the end of the year. After this, frost damage, which is worse on attacked carrots, frequently nullifies such attempts. The procedure suggested is that samples be taken, using a reliable technique, from the headland and midfield regions in the last week of September or in early

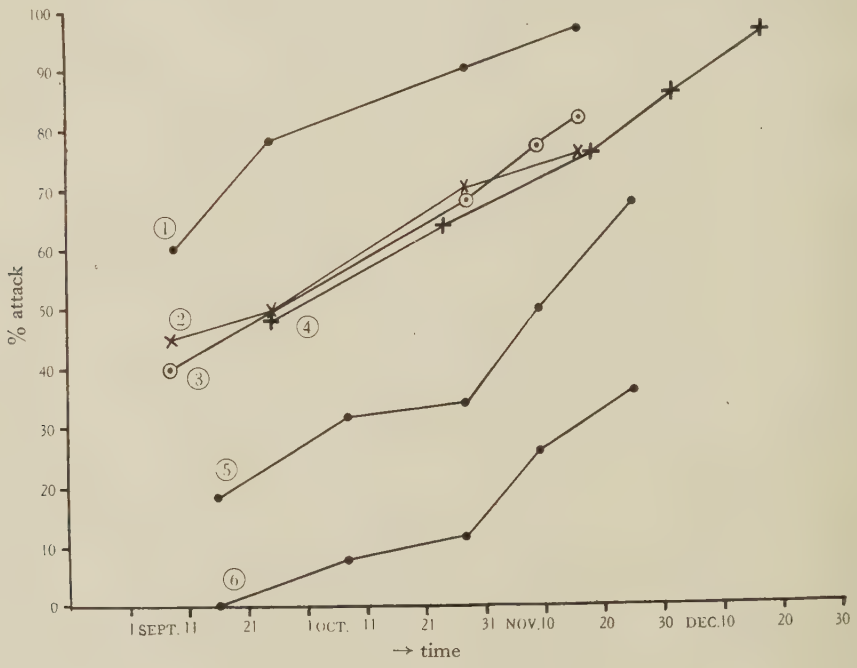

Fig. 6. Increase of percentage attack with time.

shows the increase in unsaleability with increase in the number of mines in a crop of large carrots yielding about 25–30 tons per acre. Curve 2 is the same for a crop yielding 15–20 tons per acre and curve 3 is for a light crop yielding some 5–8 tons per acre. Similar curves lying between these can be constructed for crops of intermediate weights provided that, as in the above cases, there is a full plant of carrots present. The size of the roots and not the actual crop weight is the limiting factor in unsaleability; a poor plant may give a 10-ton per acre crop and yet, as most of the roots will be large, the relationship between mines and unsaleability in that crop will be shown by curve 1. The straight lines are

October. (The dates apply to East Anglia.) With the attacks then recorded the attack/time relationship shown in Fig. 6 is then used. If on this graph, where 1 unit on the ordinate (attack) is equal to 1 unit on the abscissa (time in days), a line is drawn at 30° from the horizontal through the point obtained from a sample, the attack at later dates will fall on or near this line. The accuracy of the result will depend chiefly on sampling error, but should be correct to within ± 10% attack.

Knowing the predicted attack, it is then possible to estimate the percentage unsaleability of the crop at the level of the predicted attack. To do this the predicted percentage attack is converted into mines per

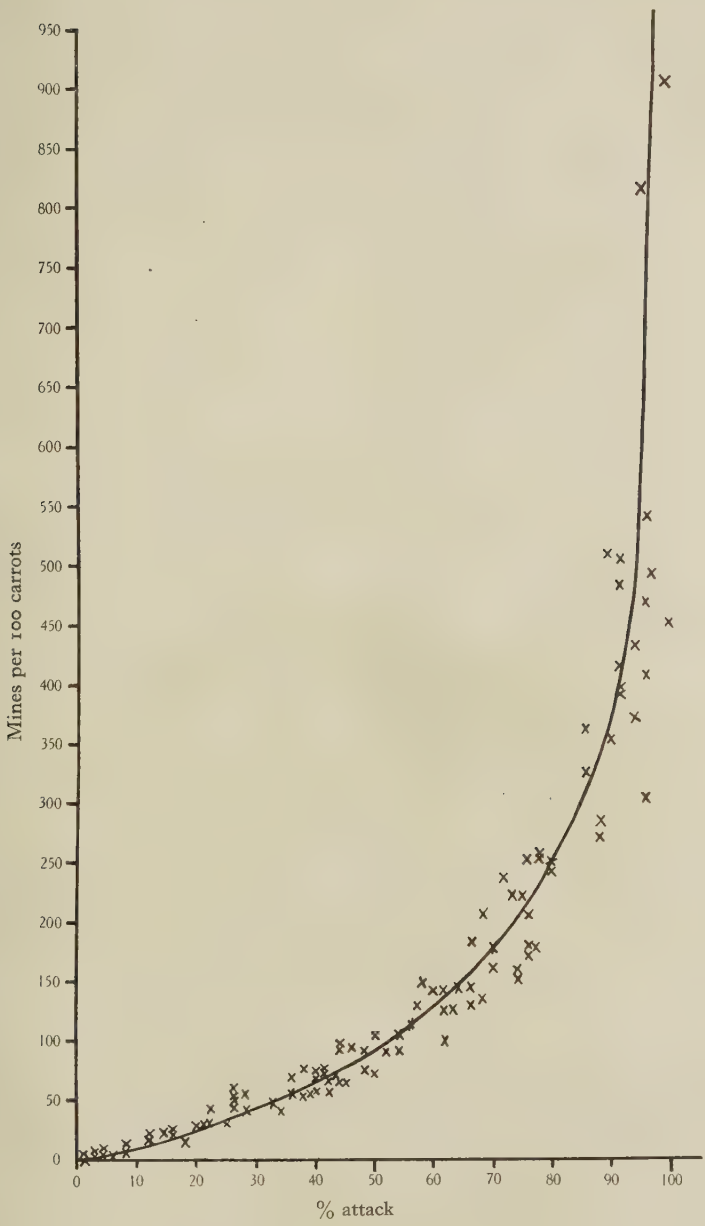

Fig. 7. Relation between mines per 100 carrots and percentage attack. (Curve drawn freehand from 104 points.)

100 carrots using Fig. 7. With this data and using Fig. 8 an estimate can be obtained of the percentage unsaleability to be expected, the curve used being related to the crop weight or average size of the carrots in the sample.

This method of prediction, constructed with data obtained in 1941, 1942 and 1943 was tested with satisfactory results during the autumn and early winter of 1944. It should assist carrot growers, who by autumn sampling could determine the order in which crops should be lifted so as to minimize losses.

mines as this is directly correlated with total larval population. Thus to compare the suitability of two or more types of shelter the corresponding headland transects are taken and graphed as mines per 100 carrots against distance from the headland. The effect of the shelter on the infestation is then given by the relative areas under each graph.

The relative suitability of two different types of headland shelter is well shown in the two lower transect curves in Fig. 1. The mines per 100 carrots for these are shown in Table 2.

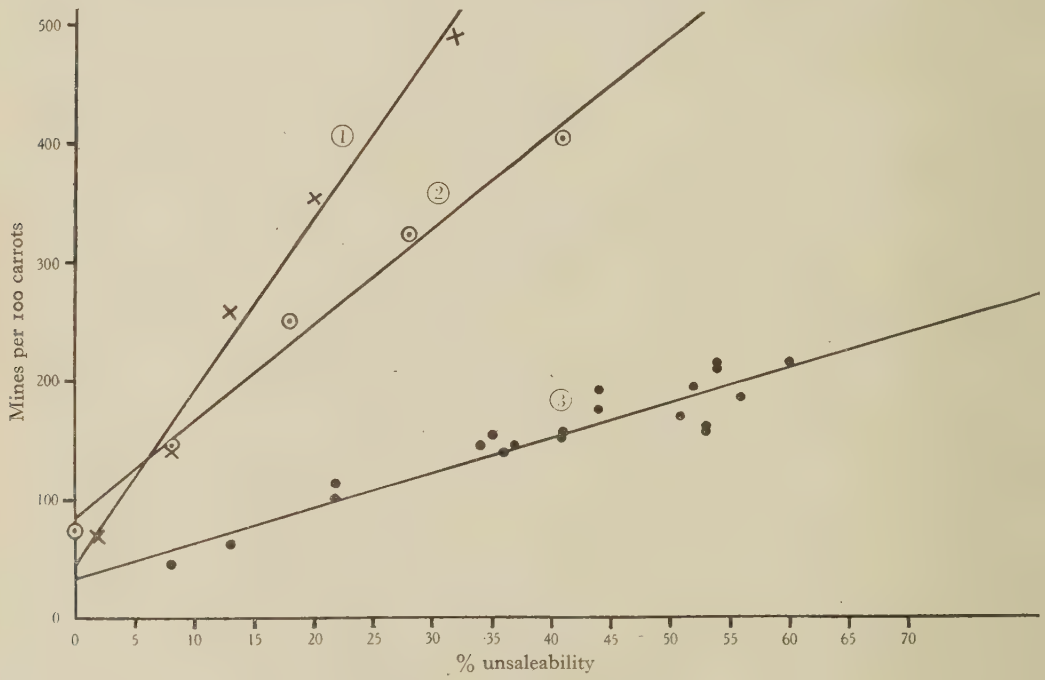

Fig. 8. Relation of mines and unsaleability.

The analysis of the attack on the field

The variation of attack over a field as shown by transects must be a direct consequence of variation in the distribution of carrot flies when oviposition is taking place. Sweeping carrot fields when flies are on the wing has shown that large numbers are present in those parts of the field where there is shelter in the form of trees, hedges and banks or ditches with a thick herb covering. Hence shelter is regarded as *one of the most important attributes of a field in determining the degree and position of the attack.* From this it follows that the most accurate and comprehensive measure of the 'suitability' of any shelter for carrot fly is the total infestation of the carrots adjacent to the shelter. The infestation is measured in

TABLE 2. *Effect of adjacent shelter on infestation*

Yards into field ...		0	5	15	30	40	50
Mines per 100 carrots	Curve II	62	56	20	12	10	0
	Curve III	4	6	2	2	2	0

The fields were both early carrots in the Swaffham (Norfolk) area, the headland of curve 3 was bordered by a trimmed hedge and that of curve 2 by a 20 ft. high hedge with thick vegetation in the bottom.

When comparing different types of shelter the examples chosen should be in the same field or locality. Each will then compete for the same carrot fly population and the environmental variables (wind, temperature, humidity and light) will then either be

constant for each shelter or be modified by the shelter alone.

An attempt can be made to eliminate the numerical differences in fly population and therefore to compare the relative suitability of different shelter types in different areas. This may be done by constructing mines per 100 carrots/distance graphs and comparing the ratios of the areas under the 0–15 and 15–50 yd. sectors of the curves. This has been done for the three headland transects shown in Fig. 1 and the comparison is given in Table 3.

TABLE 3

	Areas under the mines per 100 carrots/distance graph		
	0–15 yd.	15–50 yd.	Ratio
Curve 1	26·0	15·6	1·67
Curve 2	13·0	8·0	1·63
Curve 3	1·3	1·2	1·08

The difference between the shelter types on the headlands of curves 2 and 3 is still apparent and there is a suggestion of the equivalence of the shelter types of curve 1 and 2 (potatoes at Mepal (Isle of Ely) and high dense hedge at Swaffham respectively).

CONCLUSIONS

From this investigation it has become apparent that the pattern and intensity of carrot fly attack in a field is largely determined by the degree, position and suitability of the adjoining shelter. Certain recommendations, chiefly aimed at modifying the shelter and making it less suitable for aggregation of the carrot fly, can therefore be made. These include:

(1) Hedge bottoms should be kept free of low-growing vegetation and the hedges kept well trimmed along their sides. A tall hedge with open bottom acts quite efficiently as a windbreak, but is much less suitable shelter for carrot fly than one which is well grown up with weeds.

(2) Ditches, banks, etc., bounding carrot fields should be kept as free as possible from tall thick vegetation by cutting and burning.

(3) Potatoes provide excellent shelter and should not be grown alongside carrot crops. (See Petherbridge *et al.* (1942), *Ann. appl. Biol.* **29**, 380.)

(4) Carrots are best grown in large fields, since the headland effect extends only a limited distance out into the midfield. Briefly, the closer the headlands are to one another, the more heavily attacked is the midfield for a given total infestation.

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A study of the distribution of the swede midge (*Contarinia nasturtii* Kieffer) in Devon and symptoms of its attack on various host plants

BY D. C. THOMAS, *Seale-Hayne Agricultural College, Newton Abbot, Devon*

(With Plate 7 and 2 Maps)

A survey of swede midge damage in Devon is described, showing that the attack on cow cabbage is most severe in the north and west of the county, while attack on swedes is heaviest in the south and east of the county; the causes of this phenomenon are considered. The life history of the insect is discussed in relation to farming procedure with various crops. Short descriptions and photographs are given of the general symptoms of damage by the midge on the more important cruciferous crops.

INTRODUCTION

The damage by the swede midge (*Contarinia nasturtii* Kieffer) to cruciferous crops has long been recognized by entomologists and also to some extent by growers. It would appear, however, that in this country little investigation has been made into the distribution of the pest, its prevalence on different crops or its control on an economic scale. Mr L. N. Staniland, Advisory Entomologist, of the South Western Province, therefore suggested that this investigation could be profitably made. The final object of the work is the practical control of swede midge attacks

on agricultural and horticultural crops. A preliminary survey of the distribution of the midge in Devon on cow cabbage in 1942 suggested that certain factors determining the intensity of attack required separate investigation before the practical methods of control could be explored. The most important of these factors appeared to be the particular farming practice in relation to midge attack on various crops and the biology of the midge itself.

Farming practice and the life history of the midge

The biology of the swede midge has been investigated by a number of workers in this country (Taylor, 1912; Dry, 1915), in France (Mesnil, 1938) and in Germany (Roesler, 1937). Their accounts of the life history agree on most of the main points. The midge has three generations per year and according to Dry sometimes a fourth, while Roesler mentions a fifth generation. It is generally agreed, however, that the three summer generations of adult flies emerging in June, July and August produce the most individuals and are the generations of economic importance. The larva hibernates in a cocoon in the soil and pupates in the spring. The adults emerge in June and lay eggs in masses of 15–20 in the axils of the younger leaves of the host plant. These eggs hatch in 4–9 days, according to the temperature, and rapidly produce the characteristic symptoms of attack on the host plant.

In Devon there are the three well-marked generations in June, July, and August, respectively, and there would also appear to be an earlier generation of adults emerging in May and another emerging in late September or even early October. Thus early sown Brassicas have been observed in Devon to be attacked in May, while autumn-sown spring cabbage and late-sown rape have been found with larvae feeding on them in late September and early October. It seems reasonable, therefore, to conclude that there can be five generations per year in Devon and that this increase in number of generations is associated with the mild climate of the south-west. Moreover, these generations are not without some economic importance on certain crops, e.g. spring cabbage.

Cow cabbage is sown in autumn for planting out in early summer of the next year and it is therefore possible for the crop to receive the attention of at least five generations of midge before reaching maturity. Moreover, the majority of farmers do not raise their own plants but buy them from a limited number of growers who specialize in the production of this and other *Brassica* seedlings. Thus many of the plants come from areas which may have become centres of infection from which the midge is distributed over the country.

There are three main areas of production of cow cabbage plants on an intensive scale and these are responsible for supplying a large percentage of the crop grown in Devon. One of these areas is at Kennford, south of Exeter, another around Chittlehampton and Umberleigh, near Barnstaple, and the third lies beyond the county boundary around Launceston and Bude.

On swedes the intensity of attack is probably related to the date of sowing, earliest sown crops being the most attacked, as suggested by Dry (1915), although further information is required on this point.

On horticultural land the attack of swede midge on Brassicas is undoubtedly favoured by the repeated and intensive growing of these susceptible crops, and this is supported by Mesnil's observations on cauliflower in the Saint-Omer district of France. There seems to be little doubt that the midge is spreading and increasing in Devon on such crops and its control may easily become one of the major factors in the successful growing of these crops on horticultural land.

Table 1. *Relation of number of larvae present per plant to the subsequent production of 'many-neck' condition in swedes at Dawlish in* 1944

Larvae per plant	Plants examined	% of plants subsequently showing 'many-neck'
4–7	42	2
8–12	61	14
13–16	35	42
16	53	58

Symptoms and nature of damage on various host plants

Swedes. On this plant there are two well-known symptoms of damage by the midge. These are, first, the curling and stunting of the central leaves which may develop in less than a week after the larvae are hatched (the 'crumple leaf' condition), and, secondly, the development of secondary side shoots which is consequent upon the check to the main growing point and occurs much later. This is known as the 'many-neck' condition. Both these symptoms are adequately described and figured by Taylor (1912), Dry (1915) and Barnes (1926).

From counts made in a swede field at Dawlish in 1944 it appears that the 'many-neck' condition does not inevitably occur on all attacked plants, but only on plants carrying enough larvae to cripple the central growing point very severely. Table 1 indicates that the critical number of larvae required to produce 'many-neck' condition may be about 13–15 per plant. It is likely that this figure will vary with climatic conditions which favour or check plant growth and with the presence or absence of other

pests and diseases, particularly the cabbage aphis, *Brevicoryne brassicae*.

The effect of midge attack on the swede crops is seen later in the season, as general poor growth is a result of 'crumple-leaf' and 'many-neck', but figures are not yet available for the actual reduction in weight of the crop harvested due to midge attack.

Common turnip. This crop is much less severely attacked by the midge than are swedes, even when planted in a pure stand. When, as is not infrequent, a mixture of the two crops is planted, the difference in amount of attack is very striking.

This is probably due to the swedes being more attractive to ovipositing midges and also to the greater powers of recovery of the common turnip. The symptoms on turnips are very similar to those on swedes, but the 'many-neck' condition is rare even when many larvae are present.

Rape. Swede midge attack on rape, while by no means as common as on swedes, does occur and may sometimes affect every plant in a field. It is reasonable to suppose that in such cases an emergence of adult midges has coincided with a particularly susceptible or attractive stage of the crop. The symptoms are of severe 'crumple-leaf' and a general stunting of the plant (Pl. 7, fig. 1). 'Many-neck' condition does occur as a secondary development, but is quite unusual. Damage to the crop is not of any considerable economic importance in Devon.

Kale. The effects of midge attack appears to vary considerably on the different varieties of kale. Thus, on the ordinary farm-grown marrow-stem kale, a mild primary 'crumple-leaf' condition appears when the plant is about 6 in. high. This, however, rarely leads to any dwarfing, and the habit of growth of the plant causes crumple of the expanding and elongating leaf so that it finally becomes a small crumpled area forming a notch on the side of a well-grown and otherwise fairly normal leaf. Attack at a later stage of development has not been noticed.

On the vigorous and hardy 'Hungry-Gap' kale commonly grown in allotments and gardens the effect of swede midge attack is quite remarkable (Pl. 7, fig. 2). A severe 'crumple-leaf' condition appears to cause a systemic dwarfing of the plant, even though it may have been well grown prior to attack. A proliferation of secondary shoots occurs from the axils of the main leaves, which in due course drop off. These secondary growths are, however, themselves affected by the original attack, although they are not themselves carrying any midge larvae, and the whole plant remains deformed and stunted.

One occurrence of swede midge on Russian kale was reported from Cornwall in 1944 and here also a severe 'crumple-leaf' and some stunting are said to have occurred, but it was not possible for the author to see this particular crop.

Cabbages. The symptoms of midge attack on cabbage are a primary crumpling and distortion of the central leaves followed by an almost complete cessation of growth of these leaves, while the outside leaves continue to enlarge. This produces the most characteristic 'button-hearted' condition shown in Pl. 7, fig. 3 on savoy and in Pl. 7, fig. 4 on cow cabbage. If weather conditions are unfavourable for plant growth, the crippling of the growing point may produce permanent 'blindness' and this is particularly so in periods of drought. Thus many growers think that 'blindness' in cabbage is caused merely by lack of rain. If, however, growing conditions are good and the plant has not in the meanwhile been attacked by cabbage root fly, the central shoot will eventually recover. The final heart thus produced is, however, a very inferior product to that of the healthy plant and considerable losses are experienced by the growers, particularly with savoys, which appear to be the most susceptible of the cabbage varieties.

There is also a condition of 'blindness' in Brassicas of which the cause is not known, but it is certainly not due to swede midge. The symptoms of this are the complete absence of a growing point as compared with a plant attacked by the midge where the growing point is always visible, even if extremely dwarfed. A somewhat similar condition in cauliflower has been observed in Germany and is stated to be due to thrips (Roesler, 1936).

Spring cabbage sown in the late summer or autumn can be attacked by the last generation in the year and also again by the first generation of the next year, but it recovers rather better than do other cabbages from midge attack, probably owing to its rapid growth in spring and the smaller number of midges present in first generation.

Broccoli and cauliflower. Damage to cauliflower by swede midge has been noticed in France (Mesnil, 1938) and Germany (Roesler, 1937). In Devon, early autumn cauliflower appears to be more susceptible to attack than is late broccoli, presumably because it is available to the active and numerous flies of the summer broods. The cauliflower fails to produce any 'curd' when severely attacked, but does not always produce secondary side shoots. The damage to broccoli is very similar, except that there is frequently a very marked development of secondary shoots which do not produce any 'curds'. Pl. 7, fig. 5 shows the effect of severe midge attack on a single plant of early broccoli. The loss to the growers due to midge attack on this crop can be quite considerable in areas of intensive broccoli production.

The plants figured in the plate were attacked under field conditions and were only placed in flower-pots for convenience in photographing them.

THE DISTRIBUTION ON COW CABBAGE AND SWEDES IN DEVON

The survey of the distribution of the midge in Devon was made in the course of the general

advisory visits and wireworm survey and as will be seen from the accompanying maps tended to follow the courses of the main arterial roads in Devon. The procedure was to walk straight across the field of cabbage or swedes and back again counting approximately 500 plants in five groups of 100 each, noting at the same time the number of plants showing symptoms of midge attack in each group. Thus the distribution maps are based on symptoms of attack and not on actual numbers of larvae present. A very similar procedure was adopted by Dry in his estimations of attack (Dry, 1915).

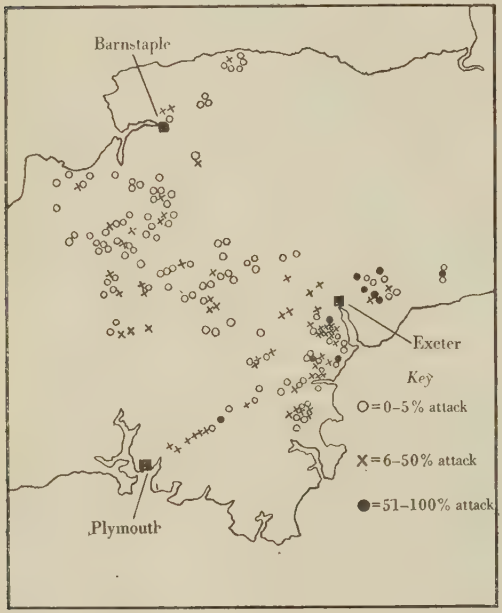
At the end of the first year's (1942) survey it became apparent that there were marked differences in the distribution of attack in the two crops examined. The heaviest attacks of midge on cow cabbage occurred in the north-west of the county in an area roughly centred around Holsworthy, while only slight attacks were observed in the south and east of the county, although a considerable acreage of cow cabbage is grown in the latter areas.

With swedes, however, the opposite obtained, the heaviest attacks being observed in the south and particularly the east of the county, while the north and the north-western areas were comparatively free from attack. It was not considered that the number of fields examined in 1942 (79 of cow cabbage and 63 of swedes) was sufficient to justify any general conclusions and the survey was continued in 1943 and 1944. In the three years 234 fields of cow cabbage and 185 fields of swedes were inspected and the same general trends of distribution were observed in each year. In 1943 there appeared to be a southern extension of the attacks on cow cabbage, but this was not maintained in 1944 when attacks on cow cabbage were much less severe everywhere than in the previous two years. It is the opinion of the Advisory Entomologist that swede midge is becoming more prevalent in the province and this is supported by the occurrence of the midge in 1944 on various crops in localities in south Devon where it had not previously been observed.

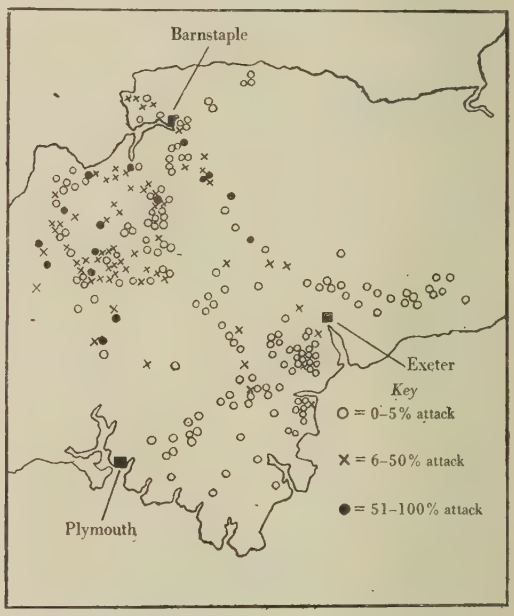
Without further detailed knowledge of the biology of the midge, it is not possible to assign exact causes for the striking differences observed in distribution of the midge on the two crops. In the light of existing knowledge, however, the following alternative explanations may be tentatively suggested:

(i) That there are biological races of the midge which are specific to the different host plants.

(ii) That the differences are produced by variation in climate and farming practice in the two areas. Thus, according to the maps contributed by the Meteorological Office, Air Ministry, British Rainfall Organization, to the Report of the Land Utilization Survey (Dudley Stamp, 1941), the average annual rainfall for the period 1881–1915 in the Kennford



Map 1. Distribution of swede midge attack on swedes in Devon in the years 1942–3–4.



Map 2. Distribution of swede midge attack on cow cabbage in Devon in the years 1942–3–4.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

areas near Exeter is approximately 35 in., whereas the corresponding figure for the area in north-west Devon, where midge attack is serious on cow cabbage, is between 45 and 60 in. It is considered that the young larvae have less protection from drought on cow cabbage than on swedes and that the drier conditions prevailing in south and east of the county do not favour the insect on cow cabbage.

In general, swedes are tilled some 2 or 3 weeks earlier in the south and east of the county than in the north and west. As was shown by Dry (1915), earlier sown swedes are more heavily attacked by midge than are later sown swedes and it is thought probable that this may account for the difference in intensity of attack in the two areas.

The distribution of the midge attack within the field was examined in several different crops. The percentage of plants showing symptoms of midge attack was determined on 250 plants on the headland and 250 plants approximately in the centre of the field. In each host crop examined the attack on the headland was consistently higher than the attack in the middle of the field. In only one field, 2 acres of cow cabbage near Bideford, north Devon, was the percentage of damaged plants higher in the centre, and this may be explained by the previous cropping history which included swedes on one-half of the field in the previous year. Table 2 shows the results of this survey of distribution within the field on five crops. It seems likely that the heavier attack on the headland may be a shelter effect, although the possibility of there being a reservoir of midge on wild cruciferous hosts in the hedge cannot be overlooked.

TABLE 2. *The distribution of swede midge attack on headlands and centres of fields of five different crops*

Host plant	No. of fields	Average % attack on headland	Average % attack in centre
Cow cabbage	54	28·4	9·5
Savoys	11	58·0	24·7
Swedes	40	21·9	15·1
Turnips	14	8·2	2·0
Rape	4	14·6	5·5

Grateful acknowledgements must be made to Mr L. N. Staniland, Advisory Entomologist, South Western Province and to Dr H. F. Barnes, Department of Entomology, Rothamsted Experimental Station, Harpenden, Herts, for their assistance and interest in this work. Particular thanks are due to Mr E. C. Large, Mycology Department, Seale-Hayne College, who is responsible for the excellent photographs of the symptoms of damage, and also to the several members of the Assistant Staff of the Entomology Department, Seale-Hayne College, who assisted in the field work.

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EXPLANATION OF PLATE 7

Fig. 1. Rape showing first symptoms of swede midge attack by *C. nasturtii*.

Fig. 2. Hungry Gap kale showing the final effect of attack by several generations of *C. nasturtii*.

Fig. 3. Savoy cabbage viewed from above showing complete stunting of central growing point by *C. nasturtii*.

Fig. 4. Cow cabbage showing crippling of centre shoot of a young plant just after planting out.

Fig. 5. Roscoff broccoli showing 'many-neck' condition —the final effect of early attack by *C. nasturtii*.

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Tyroglyphid mites in stored products. Ecological studies

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(With 4 Text-figures)

The ecology of Tyroglyphids in stored products, particularly wheat grain, was studied by examining samples taken from the material. The moisture content and the numbers of mites in each sample were determined and in many cases extensive temperature data were available.

Manitoba grain stored in bulk in one granary was repeatedly examined over a period of 15 months. Examinations of infested grain in other granaries provided corroborative data.

The chief mite pest of grain is *Tyroglyphus farinae* (L.). In imported grain it usually attacks only those parts of a bulk which have taken up extra moisture, e.g. from damp walls or from the atmosphere. *Glycyphagus destructor* (Schr.) is commonly found throughout the surface layer of a bulk of grain; it does not damage the grains. Differences were found in the distribution and population density of *Tyroglyphus*, *Glycyphagus*, and the predatory mite *Cheyletus eruditus* Schr., in relation to the moisture content of the grain.

Tyroglyphus was abundant in winter and spring, but was often reduced to very small numbers during summer and autumn. This was due to the attacks of *Cheyletus*, which was nearly always associated with *Tyroglyphus*, and maintained a moderate population density throughout the year. *Tyroglyphus* is known to multiply less rapidly as the moisture decreases; the drier conditions in the grain during summer and autumn enabled *Cheyletus* to control it. A subsidiary factor was the migration of *Tyroglyphus* into somewhat drier grain after dense populations had exhausted the available food (wheat germ). Extremely high moisture occasionally appeared to cause similar migrations. The seasonal temperature difference may also have been a factor affecting the predator/prey cycle, but if so its effect was masked by variations due to other factors.

Cheyletus was able to survive in grain too dry for *Tyroglyphus*. The predator was also associated with *Glycyphagus*, but was unable to reduce its numbers to a comparable extent. Hence *Glycyphagus* assisted the distribution and maintenance of *Cheyletus* populations. The predator is also known to attack various insects.

Grain stored in bags has a much larger area exposed to atmospheric moisture, so that infestations tend to be more widespread than in bulk grain. Concrete floors at ground level tend to transmit extra moisture to the bottom of a stack of grain, and to any loose grain on the floor, thereby providing conditions favourable to the growth of dense populations of *Tyroglyphus*. Hence it is important to keep the bags away from the floor by the use of dunnage. *Cheyletus* is less common in bagged grain and in flour.

Infestations of flour in bags were also studied. The conclusions drawn were similar to those relating to bagged grain. Mites tended to concentrate in the more superficial flour in a bag, very few penetrating more than a few cm. into the flour. This would facilitate control by fumigation. It seems possible that Tyroglyphids, or at least their eggs, may survive the processes of milling.

Dried fruits seemed to have become infested by *Carpoglyphus lactis* (L.) as a result of an increase in moisture.

The most effective means of preventing the infestation of stored products by mites is to keep them moderately dry, so that Tyroglyphids cannot live in them. Failing this, steps should be taken to prevent local increases in moisture, which increase the potential rate of multiplication of Tyroglyphids. It might be useful in certain circumstances to introduce *Cheyletus*, perhaps together with *Glycyphagus* as a source of food, into stored products, to prevent the possibility of *Tyroglyphus* being introduced without its predators, e.g. after a fumigation.

I. Introduction

The ecology of Tyroglyphids is dealt with here in relation to infested stored products in England, particularly grain and flour. There is some published information on the ecology of these mites in storage places, particularly in the U.S.S.R., and some useful investigations were carried out in England by Newstead & Duvall (1918) and Newstead & Morris (1920).

The investigations in the U.S.S.R have included a number of granary surveys, and some observations on the occurrence and distribution of mites in grain and seeds in relation to the moisture conditions in the materials. Probably the most thorough study of mites in storehouse conditions is the work of Belyaev *et al.* (1932) on the pests of oil seeds. While their investigation was primarily a survey, based on samples from 65 storehouses, they recognized several levels of population density, and in a few cases they recorded the numbers of mites in samples taken at various depths in the same bulk of material, relating

the population data to the moisture and temperature in the seeds. References to these and other papers dealing with ecological work, and a brief account of the results, have been given elsewhere (Solomon, 1943, 1944).

In general, while the more important factors governing the development of mites have been recognized, not much is known about the ways in which these factors operate under various conditions. For example, the great importance of moisture in the ecology of Tyroglyphids is well known (Solomon, 1943), but there is very little quantitative information about the rate of growth or the density of populations in storehouses, as related to moisture conditions. Again, a little is known about the conditions under which the predator *Cheyletus eruditus* Schr. (Fig. 1) controls Tyroglyphids in laboratory experiments, and there have been a number of observations on its effects on storehouse populations, but the observations are conflicting, and the interactions between predator and prey populations in storehouses have never been thoroughly investigated.

In the present work, attention has been concentrated on what appeared to be the outstanding features in the ecology of Tyroglyphids, *i.e.* on the moisture conditions in the stored materials, on the quantitative relations between these conditions and the populations of Tyroglyphids and of *Cheyletus*, and on the interactions between the populations of *Cheyletus* and those of its prey.

The name 'Tyroglyphid' is used here to cover members of the old group Tyroglyphidae *s.lat.*, including the genera mentioned below. The commoner synonyms of the specific names of Tyroglyphids mentioned in this paper (see Fig. 1) are as follows:

Tyroglyphus farinae (L.). [Called *Aleurobius farinae* by Michael (1903) and Newstead *et al.* (1918, 1920).]

Tyrophagus dimidiatus Herm. [This name has been in use for some time, although English workers have generally referred to the species as *Tyroglyphus longior* Gerv. It appears from Zakhvatkin's keys (1941) that the species is certainly a *Tyrophagus* sp., but is not *T. longior* Gerv., nor any of the other members of this genus included in Zakhvatkin's system. A description of the species was given by Jary & Stapley (1937), who used the name *Tyroglyphus dimidiatus* Herm. (*longior*) Gerv.; their paper included a note on the variety *castellanii* (Hirst).]

Glycyphagus destructor (Schr.). [This is the species referred to as *Glycyphagus cadaverum* Schr. by Newstead & Duvall (1918) and later English workers. However, it appears identical with *Glycyphagus* (*Lepidoglyphus*) *destructor* (Schr.) Ouds. in Zakhvatkin's system (1941), and as he applies the name *G. cadaverum* (Schr.) to a different species, it seems necessary to discontinue the former usage.]

Gohieria fusca (Ouds.). [Syn. *Ferminia fusca* (Ouds.), *Glycyphagus fuscus* Ouds.]

Carpoglyphus lactis (L.). [Syn. *Carpoglyphus anonymus* (Haller), *C. passularum* Rob.]

Caloglyphus rodionovi Zakhv. [Syn. *Tyroglyphus mycophagus* Schulze, 1924.]

Thyreophagus entomophagus (Lab.). [Syn. *Histiogaster entomophagus* (Lab.), *Monieziella entomophaga* (Lab.).]

The main results of a number of examinations of infested materials are stated and discussed in the following Sections. Space does not permit the inclusion of descriptive accounts of the separate cases, except for one example, described in Section VII.

II. METHODS

The methods used in detecting the presence of mites, in taking samples of the infested materials, and in estimating the numbers of mites in the samples, have been described elsewhere (Solomon, 1945). The figures for population density include all stages of the species concerned, from egg to adult. While some of the materials examined were found to be free of mites, no data from these examinations are included in this paper. But when samples of infested materials were taken, a few samples of the adjacent uninfested material (if any) were always taken for comparison.

The m.c.* of grain was determined by grinding the sample in a coffee mill, weighing out three aliquots and drying in an oven, ventilated by a fan, for 4½ hr. at 115° C. Flour samples were similarly treated, except that grinding was omitted and the material was dried for only 3 hr. The m.c. of the samples of dried fruits was estimated by Dr E. E. Turtle, using a special method (Brown, 1938). All m.c. figures are given as % total weight, not % dry weight.

The temperature in flour and in bagged grain was determined with mercury thermometers, left in position at least 30 min. to equilibrate. For most of the temperature data for the bulk grain I am indebted to Mr T. A. Oxley, who used specially designed thermocouple equipment (Oxley & Henderson, 1944).

For almost all of the samples of grain, an estimate was made of the amount of damage done by the mites. Tyroglyphids consume the embryo of the grain, until the whole germ is destroyed and only a cavity remains. The endosperm is rarely attacked. The appearance of germs in various stages of destruction by mites is easily recognizable and the type of damage is readily distinguished from that caused by insect pests. In assessing the damage done in a sample, a number of grains were examined under a low-power binocular microscope, and the appro-

* The contraction 'm.c.' is used for 'moisture content' in this paper.

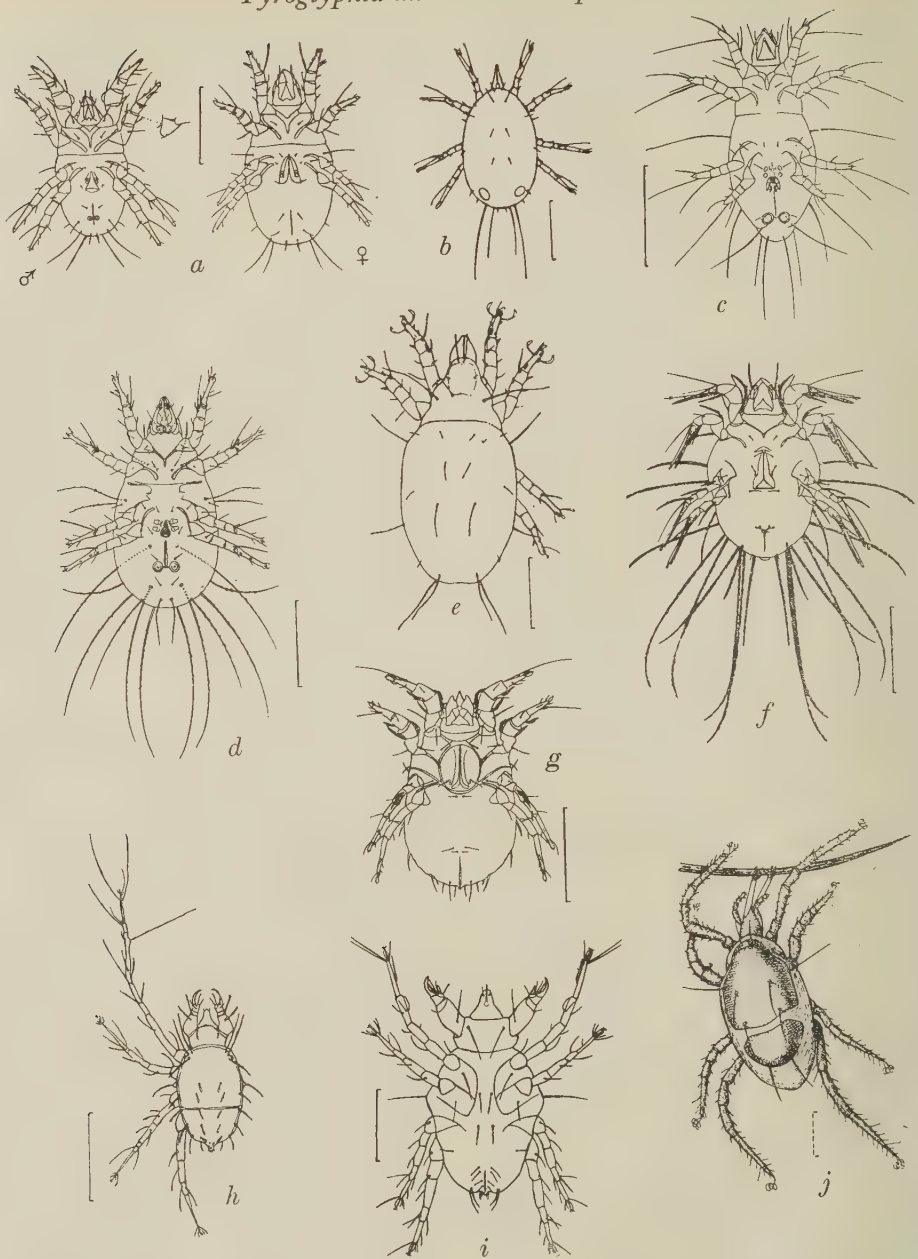


Fig. 1. Mites referred to in the text. With each figure, a 0·2 mm. line is drawn to scale.

a. Tyroglyphus farinae (L.), ♂ (left) and ♀ ventral. (From Newstead and Morris, 1920.) *b. Carpoglyphus lactis* (L.), dorsal. (From H. Weidner, *Bestimmungstabellen der Vorratsschädlinge*, 1937. Jena: G. Fischer.) *c. Thyreophagus entomophagus* (Lab.), ventral. (From Newstead & Morris, 1920.) *d. Tyrophagus dimidiatus* Herm., ventral. (From Jary & Stapley, 1937.) *e. Caloglyphus rodionovi* Zakhv., ♂ dorsal. (From Zakhvatkin, *Wiss. Ber. moskau St. Univ.* 1937, **3**, 169.) *f. Glycyphagus destructor* (Schr.) ♀ ventral. (From Newstead & Morris, 1920.) *g. Gohieria fusca* (Ouds.), ♀, ventral. (From Newstead & Morris, 1920.) *h.* The Cheyletid mite, *Cheletomorpha venustissima* (Koch), ♀, dorsal; right legs removed. (From Vitzthum, *Acari*, in *Die Tierwelt Mitteleuropas*, **3**, 1929. Leipzig.) *i. Cheyletus eruditus* (Schr.), ♀ ventral. (From Newstead & Morris, 1920.) *j.* A typical Gamasid mite; dorsal. (From Berlese, *Gli Insetti*, **2**, Fasc. 1–3, 1912. Milan.)

priate symbol in the following list selected to indicate the degree of damage in that sample:

(*a*) No mite damage.

(*b*) Minority of grains attacked; no germs completely destroyed.

(*c*) Majority of grains attacked; no germs completely destroyed.

(*d*) Majority of grains attacked; minority of germs completely destroyed.

(*e*) All (or nearly all) grains attacked; majority of germs completely destroyed.

(*f*) All (or nearly all) germs completely destroyed.

The implied correlation between the number of grains attacked and the severity of the attack on each grain was based on experience in the examination of damaged grain, and was found to hold good in the great majority of cases.

Mite infestations, like other populations, are continually growing or dwindling. Where only one examination was made, efforts were made to relate the data to the probable course of events before and after the examination. It was usually possible, for example, to detect different stages in the development of the populations in different parts of the infested bulk of material. Even populations which had died out left clear indications of their previous existence, in the form of mite remains and (in grain) the characteristic damage to the embryo. The comparison of the states of the populations in different storage places also helped to build up a dynamic picture of their ecology. However, there are obvious limitations in all these methods. Hence, special attention was paid to the work in a granary which was examined at intervals over a considerable period (Section VII). The results of this study of the development of populations provided a more or less connected account, in the light of which the data from single examinations in other granaries were more readily understandable. The figures for population density and m.c. in the samples are not given in full, but are summarized in section III C.

III. Populations in stored wheat grain

The wheat examined was all imported grain, and nearly all Manitoba No. 1. Its m.c. when first put into storage was below 13%, i.e. low enough to prevent the development of heavy infestations, and often below 12%, which is too dry to support Tyroglyphids. Such grain becomes heavily mite-infested only after taking up additional moisture.

A. *Bulk wheat*

Besides the repeated observations in the Gloucestershire granary, some of which are described in section VII, single detailed examinations were made in another Gloucestershire granary and in three London granaries. Samples of bulk grain from other storage places were also studied. The main conclusions were as below.

When grain is stored in bulk, the inner parts are protected by the outer layers from external moisture. Hence increases in m.c. tend to be more or less localized and superficial. They may come about in various ways: (i) The exposed surface of the bulk takes up moisture from the atmosphere. This process may be hastened by fine rain or mist drifting in through open windows. Wooden bulkheads allow moisture from the atmosphere to penetrate laterally into the bulk. (ii) Grain in contact with a damp wall takes up extra moisture which gradually penetrates into the bulk for a short distance (Fig. 4). (iii) Grain in contact with damp flooring, or flooring which gradually transmits moisture, as concrete often does, becomes increasingly moister. (iv) Defective roofing may allow rainwater to reach the grain. (v) Temperature differences within the bulk result in a transference of moisture from warmer to cooler parts, where it slowly increases (Anderson *et al.* 1943). Owing to the rate of heat transference through grain (Oxley, 1944), the inner parts of a bulk change relatively little in temperature, while the outer parts become warmer or cooler in accordance with the surrounding temperature (Oxley & Howe, 1944). Local heating is produced by insect infestations (Oxley & Howe, 1944) and probably to a minor degree by mites.

Distribution of mites in relation to physical conditions

Owing to the restricted distribution of the moister grain, the distribution of *Tyroglyphus* and *Glycyphagus*, and of the predator *Cheyletus*, was correspondingly restricted; they did not penetrate throughout the bulks. There was a correlation between population density and the m.c. of the grain, gradients of population density coinciding, on the whole, with gradients of m.c.

In some of the moister, infested sections there was spontaneous heating of the grain, with a temperature gradient corresponding approximately to the moisture gradient. In such cases, the raised temperature would no doubt have caused the Tyroglyphids to multiply more rapidly. But in places where there was no marked temperature gradient, the correlation between population density and moisture content was still clear. Moreover, in the winter months the temperature gradient near the surface of the bulk was in the opposite direction to the moisture gradient, and in such cases the population density was clearly related to the moisture content, and not to the temperature.

It seemed therefore that the effect of temperature on the population density was of secondary importance and was generally masked by the effect of moisture.

2. Relationship between Tyroglyphids and predators

An unexpected fact was observed in the Gloucestershire granary in the late summer of 1940, when the populations of *Tyroglyphus* disappeared almost completely. The sets of samples from some of the places where the infestation had been well established now contained no living *Tyroglyphus* at all; in other such places a few individuals were found in the samples.

This striking decline could not be attributed simply to physical changes. The maximum temperatures encountered in the grain were very far below those required to kill Tyroglyphids. The m.c. of the grain commonly remained as high as 14, 15 and even 16% in parts of the previously infested sites. The decline could, however, be attributed to the presence of fair numbers of the predatory mite, *Cheyletus*. The numbers of this mite increased in the summer months, and although it never reached densities comparable with those of well-established *Tyroglyphus* populations, it was probably sufficiently numerous to destroy the latter.

Towards the end of the year, with the onset of winter conditions, *Tyroglyphus* populations reappeared (although numbers of *Cheyletus* remained), only to be destroyed again in the following summer. The same phenomenon was observed in all the infested parts of the grain which were studied (see Table 3, p. 96), and examinations of other infested granaries provided data in keeping with the above.

An example of this recurrent annual cycle is described in detail in section VII.

It seemed that the operation of the cycle could best be explained as the result of the different effects of moisture, and perhaps temperature, on predator and prey—high moisture (and perhaps low temperature) favouring *Tyroglyphus*, lower moisture (and perhaps higher temperature) favouring *Cheyletus*. An important secondary factor was the observed tendency of *Tyroglyphus* to move from the dampest grain after a time, apparently because of exhaustion of the wheat germ, and perhaps also in some cases because of unfavourable effects of extremely high moisture. In the drier grain to which *Tyroglyphus* migrated, it was more readily exterminated by *Cheyletus*.

Cheyletus also occurred in association with *Glycyphagus*, and certainly preyed upon it. However, there was no evidence that the predator ever reduced large *Glycyphagus* populations to very small numbers, probably because the rapid movements and long hairs of *Glycyphagus* afford it considerable protection. But the fact that *Cheyletus* is apparently able to maintain itself on populations of *Glycyphagus* is important in another respect. It assists the wide distribution of *Cheyletus* over the bulk, so that it must often be already present, or close at hand, when a population of *Tyroglyphus* becomes newly established. Since *Glycyphagus*, moreover, does not attack the grain itself, and seldom reaches great densities, it must be regarded as a beneficial mite, at least in grain.

One or more species of Cecidomyiid flies, the larvae of which are predators of *Tyroglyphus*, were found in moderate numbers on a few occasions, but there was no evidence of decisive effects on the *Tyroglyphus* population. Small numbers of Gamasid mites, which are also predators on Tyroglyphids, were found fairly frequently, but there were too few of them to have much effect on the numbers of *Tyroglyphus*. Psocids, which possibly attacked the eggs of the mites, were also found occasionally, but again they were not common nor abundant enough to play an important role. (For references to papers on Cecidomyiids and Gamasids as predators of Tyroglyphids, see Solomon, 1943, 1944.)

The ability of *Cheyletus eruditus* Schr. to destroy dense populations of *Tyroglyphus* has been recorded earlier by Ewing (1912; the species he studied was probably *C. eruditus* Schr.), and by Sigaard (1920). Other workers have stated that *Cheyletus* had little effect in reducing *Tyroglyphus* populations, e.g. Newstead & Duvall (1918), Rodionov & Furman (1940). The latter authors came to this conclusion with reference to grain, and held that *Cheyletus* was even less successful in finer materials.

In view of the conclusions in the earlier part of this section, it seems likely that the divergence of opinions may have been due to differences in the physical conditions in the materials observed.

There are, in fact, several references in the literature to the influence of physical conditions on the effect produced by predators. Newstead & Duvall (1918) wrote: 'Experiments...with small quantities of wheat have demonstrated that the presence of *Cheyletus* is ineffectual to prevent the rapid multiplication of *Aleurobius* [*Tyroglyphus*] *farinae* under favourable conditions of temperature and moisture for the latter.' Referring in general to the mites predatory on Tyroglyphids, Shepard (1932) observed that the predators had least effect when there was enough moisture for rapid development of the Tyroglyphids, and most effect in drier conditions. In some cases the Tyroglyphids almost disappeared (Shepard, 1939).

On the other hand, Sigaard (1920) noticed a periodicity between dense populations of Tyroglyphids and the subsequent increase in numbers of *Cheyletus eruditus* Schr., which almost exterminated them.

The present work shows the connexion between these two sets of observations, and relates the periodicity to the physical factors.

B. *Bagged wheat*

Single detailed examinations of storages of wheat in bags were made in two buildings in Devonshire

and in a large building in Yorkshire. The following are the main conclusions.

Wheat stored in bags has a much greater area exposed to the atmosphere than has bulk grain. Any protection afforded by the bagging material is slight and temporary. Since there are air spaces in a stack of bagged wheat, atmospheric moisture can circulate to some extent between the bags. Similarly, moisture from a damp wall or floor may penetrate into the depths of a stack by convection through these spaces. If the grain is dry when bagged, moisture penetrates slowly from the outside inwards, so that a gradient is set up in each bag of grain.

Because of the increase in the exposed area, and the consequent extensive uptake of moisture, infestations tend to be more widespread in bagged grain than in bulk.

When stored on a concrete floor at ground level, the bags should be well raised from the floor on strips of timber at least 10 cm. high. This prevents the direct passage of moisture through the concrete into the wheat, by providing a space in which air can circulate and carry away much of the excess. Where such steps had not been taken, dense populations of *Tyroglyphus* tended to develop in moist grain near the floor, and spread upwards into the stack. (A similar tendency was noted in bulk grain standing on concrete.) In one of the granaries inspected, a single layer of tarpaulins had been laid beneath stacks of wheat on a concrete floor, but was found to have been insufficient to prevent moisture passing through to the grain.

In the three storages of bagged grain which were examined, *Cheyletus* was less prevalent than it was found to be in bulk grain. This may have been fortuitous, but it is possible that the bagging material may tend to exclude *Cheyletus*, which is considerably larger than *Tyroglyphus*.

Observations in one of the stores showed that the infestation of suitably moist grain may be delayed if this grain is far removed from the main centres of infestation.

C. *Collected data from stored wheat*

In this section, consideration is given to the collected data from all the samples taken from the granaries and other stores examined.

1. *Moisture content*

The 668 samples are divided into six groups, on the basis of their m.c., as shown in Table 1. None of the samples had a m.c. less than 11%; in more than half of them it was between 12·0 and 14·0%.

It must be emphasized that the sampling was in most cases restricted to the vicinity of infested grain. Usually only a few samples were taken from the normal, uninfested grain near the infested parts (e.g. see Fig. 4). Since the infested grain was nearly always moister than the main bulk, the m.c. distribution indicated in Table 1 is different from that which random sampling would reveal. The high proportion of samples with m.c. about 13% is due to the selective method of sampling. Random sampling of the same grain would show a high peak between 11 and 12% m.c., with a fair number of samples between 12 and 13%, and very few moister than this.

2. *Frequency of occurrence and population density of mites, in relation to moisture content*

The frequency of occurrence of *Tyroglyphus*, *Glycyphagus* and *Cheyletus* in the samples of the different m.c. groups is shown in Table 1. In Fig. 2, the points indicate, for each group, the percentage of the samples infested by each of the three species. This arrangement of the data eliminates the chief effects of the differences in the numbers of samples in the six groups. The position of each point along the m.c. scale indicates the arithmetic mean of the m.c.'s for the group of samples concerned.

The summarized data on the population density in the samples are shown in Table 1 and Fig. 3. Since populations increase by multiplication, different levels of density are best treated as steps in a geometric series, not an arithmetic series. For each group of samples, therefore, the best measure of the average level of population density for the group is the geometric mean of the densities in the individual samples, not the arithmetic mean. The population density figures in Table 1 are geometric means. The samples are arranged in six m.c. groups, as before. The mean densities are calculated for each species separately, and only the samples containing *Tyroglyphus*, e.g., have been considered in calculating the mean densities for that species. The resulting mean values of the population densities of the three species in each of the six groups of samples are plotted in Fig. 3. As in Fig. 2, the positions of these points along the m.c. scale indicate the arithmetic means of the m.c.'s of the samples concerned.

(*a*) Tyroglyphus *and* Glycyphagus. The following points may be established from Table 1 and Figs. 2 and 3:

(1) More than one-third of the samples infested by *Tyroglyphus* had a m.c. of 13·0–14·0%. Moister grain was less common, drier grain less frequently infested (Table 1). The same applies to the data for *Glycyphagus*.

(2) *Glycyphagus* increased sharply in frequency as the m.c. increased up to 14·0–15·0%, and declined again as the m.c. further increased (Table 1, Fig. 2). Fig. 3 shows there was an increase in population density as the m.c. increased up to 14·0–15·0%, but very little increase in the moister samples in which *Glycyphagus* occurred. It is possible that these higher moistures are less favourable, or no more favourable, to *Glycyphagus* than the 14·0–15·0% range; but it also seems likely that food shortage

becomes a limiting factor at a certain level of population density, since *Glycyphagus* lives on dust and fine particles in the grain, and it is noticeable that samples heavily infested by this species often yield no fine vegetable matter on sieving.

(3) *Tyroglyphus* occurred more frequently and in greater density as the m.c. increased. The slight reduction in frequency at m.c.'s above 16% (Fig. 2) can be attributed to the increased destruction of the wheat germ by the mites at high humidities (leading to migration or starvation) and to the occurrence of some extremely moist rotten grain in which *Tyroglyphus* could not live. *Tyroglyphus* occurred in much greater density than *Glycyphagus* and *Cheyletus*, this discrepancy increasing as the m.c. increased.

a continuous upward trend with rising m.c., reflecting the density trend of its chief prey, *Tyroglyphus* (Fig. 3). The frequency diagram (Fig. 2), on the other hand, shows two important differences between the distribution of *Cheyletus* and that of *Tyroglyphus*:

(1) *Cheyletus* occurred more frequently than *Tyroglyphus* and *Glycyphagus* when the m.c. was 12·0–13·0%. It also occurred fairly frequently in grain of m.c. (11·0–12·0%) too low to support the others. In most of such cases it seemed that *Cheyletus* had wandered from adjacent, moister grain containing *Tyroglyphus* or *Glycyphagus*; populations of *Cheyletus*, being self-predatory, are able to survive for some time in the absence of other prey. In a few

TABLE 1. *All the samples of wheat grain sorted into six groups according to moisture content. For each group, the table shows the number of samples falling into it, the number (and %) of these samples containing* Tyroglyphus, *and the geometric mean population density of* Tyroglyphus *in the samples in which it occurs. Similar data are given for* Glycyphagus *and* Cheyletus. *The frequency data are plotted in Fig.* 2, *and the population density data in Fig.* 3.

Moisture content ...	11·0–11·9%	12·0–12·9%	13·0–13·9%	14·0–14·9%	15·0–15·9%	16% and over	Total
No. of samples ...	101	200	170	83	51	63	668
Samples containing *Tyroglyphus* (and the same as % of total)	0	13 (6·5%)	66 (38·8%)	40 (48·2%)	26 (51·0%)	32 (50·8%)	177
Geometric mean density in these samples. (No. of *Tyroglyphus*/100 c.c. grain)	0	20·84	212·8	937·9	859·0	6,980	
Samples containing *Glycyphagus* (and the same as % of total)	0	37 (18·5%)	101 (59·4%)	57 (68·7%)	28 (54·9%)	6 (9·5%)	229
Geometric mean density in these samples. (No. of *Glycyphagus*/100 c.c. grain)	0	11·13	40·94	83·10	92·92	96·23	
Samples containing *Cheyletus* (and the same as % of total)	35 (34·7%)	79 (39·5%)	95 (55·9%)	45 (54·2%)	21 (41·2%)	25 (39·7%)	300
Geometric mean density in these samples. (No. of *Cheyletus*/100 c.c. grain)	8·65	10·87	19·31	57·24	89·18	213·9	

It should be emphasized that the samples were taken from populations of various ages, under varied conditions of storage and of temperature, that the predator *Cheyletus* was in various stages of interaction with its prey or absent altogether, and that in some of the moister samples the mites had largely migrated or died out after consuming the available food. In spite of these variable factors, the data show a clear general correlation between the population density and the m.c.—a commentary on the dominant role played by moisture in the existence of Tyroglyphids.

(*b*) Tyroglyphus *and its predator*, Cheyletus. As might be expected, the density of *Cheyletus* showed

cases, there was evidence that *Cheyletus* was preying on Psocids, and it is known to be an occasional predator of other grain insects.

(2) There was a decline in the frequency of occurrences of *Cheyletus* at higher m.c.'s beginning at 13·0–14·0 or 14·0–15·0%, in spite of the increased frequency of *Tyroglyphus*. Taking this and the first point together, it seems clear that *Cheyletus* is adapted to a lower m.c. range than that of *Tyroglyphus*, although the two ranges largely overlap.

To what extent do these data support the conception of the *Tyroglyphus*/*Cheyletus* cycle put forward above (p. 86)? The lower m.c. range of *Cheyletus* suggests that drier conditions favour the

predator. On the other hand, *Cheyletus* occurred in the greatest numbers at the higher m.c.'s. However, its effectiveness in controlling the numbers of *Tyroglyphus* depends, not on the absolute numbers of the predator, but on its numbers relative to those of its prey. Fig. 3 illustrates the fact that the population and was reduced to low numbers as a result of food shortage were excluded. The same factor had no doubt operated to some extent in some of the remaining samples, and, of course, the effect of the predator on the numbers of *Tyroglyphus* was not eliminated. In spite of this, the data show that the

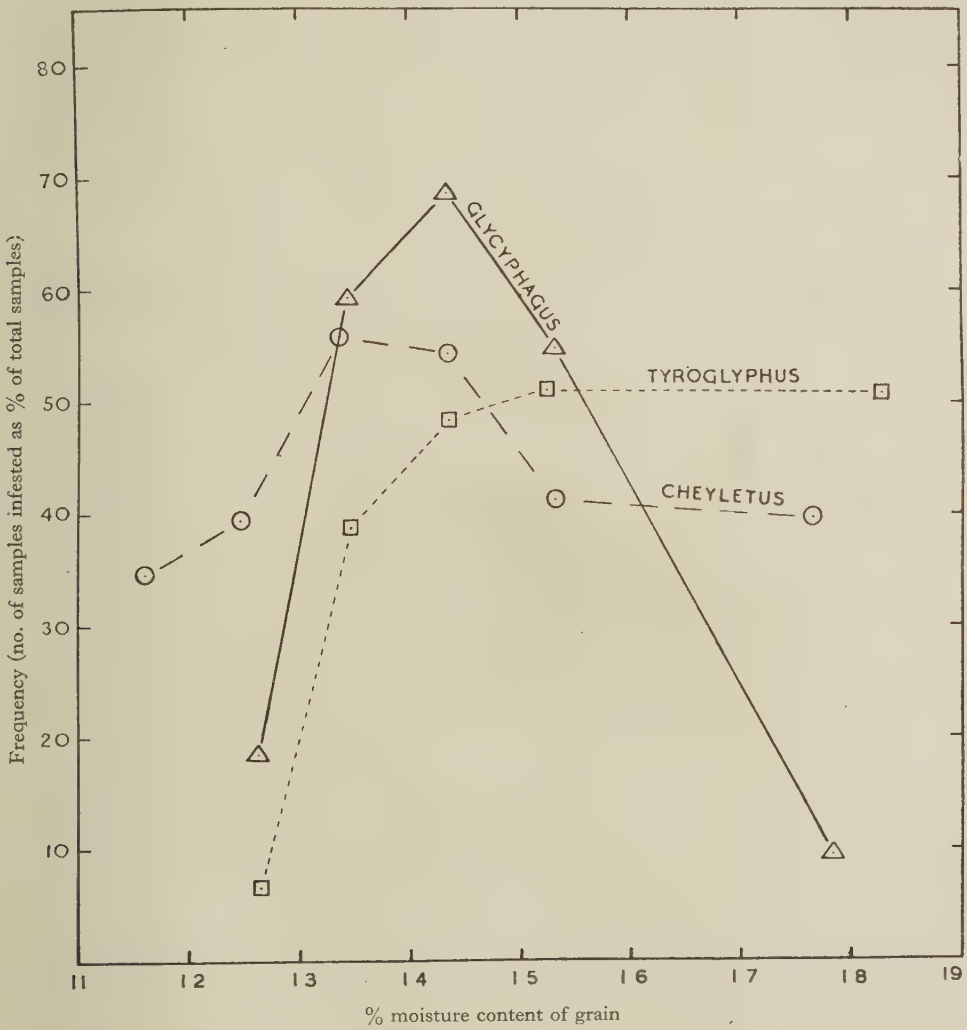


Fig. 2. Frequency of occurrence of *Tyroglyphus*, *Cheyletus* and *Glycyphagus* in 667 grain samples from various granaries, in relation to grain moisture content. For explanation and discussion, see Table 1 and text.

density of *Cheyletus*, relative to that of *Tyroglyphus*, declines markedly as the m.c. increases.

A more rigorous test of this point is shown in Table 2. Here, only the samples containing living mites of both species were selected; samples in which *Tyroglyphus* had destroyed all the embryos population density of *Tyroglyphus*, in relation to that of *Cheyletus*, became increasingly greater as the m.c. increased.

The fact that *Tyroglyphus* does multiply with greatly increasing rapidity as the m.c. rises, is shown by figures published by Zakhvatkin (1941) and

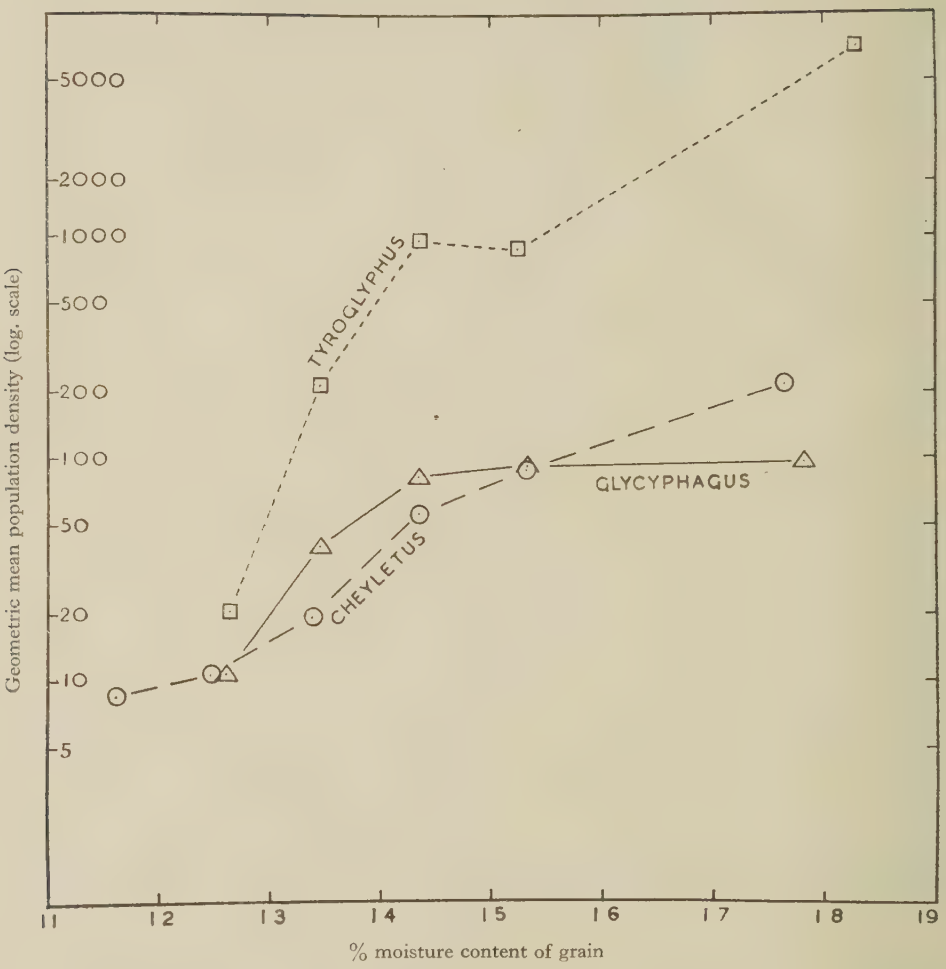


Fig. 3. Population density of *Tyroglyphus*, *Glycyphagus* and *Cheyletus* in samples from various granaries, in relation to grain moisture content. For explanation and discussion, see Table 1 and text.

TABLE 2. *Population density of* Tyroglyphus *relative to that of the predator,* Cheyletus, *at different levels of grain moisture content. Data from samples in which both species were present*

Moisture content %	No. of samples	Arithmetic mean density: Tyroglyphus (DT)	Arithmetic mean density: Cheyletus (DC)	DT/DC
12·0–12·9	4	25·7	142·2	0·18
13·0–13·9	30	943·3	119·1	7·92
14·0–15·9	18	5,193·9	287·5	18·07
16·0 and over	10	39,960·0	495·4	80·66

quoted in full elsewhere (Solomon, 1943). From these figures, it is possible to calculate the ratio of the rates of multiplication at 90 and 80% relative humidity, for four temperatures, 7, 13, 22 and 25° C. The arithmetic mean of these four ratios is 3·0, i.e. the rate of multiplication was on an average three times as great at 90 as at 80% relative humidity.

In brief, the hypothesis that drier conditions enable *Cheyletus* to control *Tyroglyphus*, while moister conditions enable *Tyroglyphus* to increase in spite of the predator, is supported by the fact that *Cheyletus* tends to occupy a lower m.c. range than *Tyroglyphus* (although the two ranges overlap for the most part),

and also by the fact that the population density of *Tyroglyphus* by far outstrips that of *Cheyletus* as the m.c. increases, particularly above 16% m.c.

3. *Frequency and density of mites, in relation to temperature*

The temperature factor, which may have affected the seasonal *predator/prey* cycle, has not been considered above. The original temperature of about three-quarters of the samples was known well enough to enable their arrangement into groups covering successive intervals of 5° C. Of these samples, those containing *Tyroglyphus* showed a good inverse correlation between m.c. and temperature, as also did those containing *Cheyletus*. This was merely an expression of the seasonal changes in physical conditions.

The (arithmetic) mean temperature for the samples containing *Tyroglyphus* was calculated, and also that for the samples containing *Cheyletus*, but no significant difference was found.

Similarly, the slight correlations found between the density of either species, and the temperature, did not provide a sufficient basis for any conclusions (see also p. 86).

Newstead & Duvall (1918) considered that mites were most plentiful in wheat during summer and autumn, due partly to the higher temperature, but it appears that they were referring to grain of unusually high m.c. In imported grain, the reduction in m.c. in summer is likely to nullify the effects of raised temperature, particularly when *Cheyletus* is present, so that the seasonal changes in the numbers of mites are often in the opposite direction to that stated by Newstead & Duvall.

IV. Flour

Storages of infested flour in Kent, Leicestershire and the Newcastle area were examined in detail. The following main conclusions were drawn.

As in grain, *Tyroglyphus* is the chief mite pest; *Gohieria fusca* (Ouds.) is common, but generally does not reach great numbers.

The degree of infestation of flour is related to its m.c., but since flour usually has a m.c. of at least 14%, moisture is not a factor limiting the spread of mites through a bulk, to the same extent as in grain. There are, however, other limiting factors in the case of flour, as mentioned below.

Besides a correlation between mite population density and the m.c. of the flour, certain other general tendencies have been observed.

Usually, there are clear indications that the infestation of a stack of flour has begun at or near the floor, and spread upwards. There are several common reasons for this, the most obvious being that the mites often come from the remnants of old infestations which have been left on the floor of the building, or are introduced on to the stack at a low level by rodents. Secondly, concrete floors at ground level are frequently a source of extra moisture, which reaches the bottom of the stack and gives rise to conditions very favourable to mites. Thirdly, loose flour always escapes from the bags on to the floor, where it frequently takes up extra moisture from the flooring or as a result of exposure to the atmosphere; this provides a very favourable breeding ground for mites, which later migrate in large numbers to the stack itself. Loose flour on the outsides of bags also tends to be particularly heavily infested, if the atmosphere is moist enough to raise the m.c. of the exposed material.

There is another reason for the comparatively heavy infestation of loose material, namely the operation of deterrent factors which hinder the penetration of mites into the depths of a bag of flour. It may be that the mechanical barrier of the bagging has a partial deterrent effect, but mites certainly do penetrate it. No doubt very closely woven material is a more effective barrier than coarser bagging, a point which may be of practical importance. But even when the more superficial flour, just inside the bagging, is heavily infested, the population density usually grades off rapidly as one penetrates into the flour, so that very few mites may be present at a depth of 4–5 cm. Sometimes they penetrate deeper than this, but they are seldom found at the centre of a bag. This applies even when there is no gradient of m.c. in the flour. The variation in the depth of penetration no doubt depends on the m.c. and the age of the infestation, but the tightness of packing and the degree of lumping together of the flour are also likely to be significant. It seems clear that the mechanical resistance of the flour to penetration is one of the deterrent factors. For example, long-haired mites such as *Glycyphagus* are never serious pests of flour in bags, although they are sometimes abundant in thin layers of loose flour outside.* There are other factors which have not been analysed.

For example, if mites are thoroughly mixed up with flour in a glass jar, and the material left to stand for 24 hr., the mites are found to have concentrated against the glass and at the free surface. This happens even in the dark, so that if light is a factor it is not the only one. A few gas samples were taken in one of the stores from two bags in which the peripheral concentration of the mites was marked, by means of evacuated flasks. The carbon dioxide concentration in these samples was below 0·05% at 4 cm. depth, and below 0·2% at 15 cm. depth. It appeared from this that the carbon dioxide concentration was not high enough to influence the distribution of the mites.

* This point was noted by Rodionov (1937), who also remarked that, for the development of dense populations, flour was a less favourable medium than groats, which allowed greater freedom of moment to mites.

Whatever the causes of it, the fact that mites tend not to penetrate beyond the superficial parts of flour in bags is of practical importance, since it enables effective fumigation to be carried out, often without the necessity of ensuring that the fumigant penetrates into the centres of the bags.

As has been pointed out elsewhere (Solomon, 1945), the eggs, at least, of Tyroglyphids are able to pass through the bolting silks used in flour mills. It is possible that mites in the hollowed-out embryos of infested grain may survive the grain-cleaning processes and the subsequent milling processes. Alternatively, mites in the milling machinery may be drawn into the flour stream and survive in the finished product. However, these possibilities have not been specifically investigated, and my examinations of stored flour have not given conclusive proof of such occurrences.

Although one lot of infested flour (in Kent) had a high acidity (*p*H 4·16 instead of the normal 6·0), acidity tests on other samples of infested flour have not revealed any marked deviations from the normal. (I am indebted for the acidity data to Dr J. D. Mounfield, of Cereals Research Station, St Albans, who collaborated in the investigation of the Kent storage.)

V. Other products

A parcel of infested oats stored in bulk in Devonshire was found to have an unusually rich mite fauna associated with high temperature and moisture conditions. At 15 cm. depth, where the temperature was 47° C. and moisture content 17·9%, there were numbers of *Caloglyphus* (? *rodionovi* Zakhv). Nearer the surface, the same species was present together with a Eupodid, *Tydeus* sp., the predators *Cheletomorpha venustissina* (Koch) and *Cheyletus eruditus* Schr. and several species of Gamasids. In the deeper grain, where the temperature was 48–53° C. and m.c. 14·7–15·6%, there were no living mites.

A large storage of dried fruits in South Wales was examined. Raisins with a high m.c. of 23–27% were heavily infested by *Carpoglyphus lactis* (L). (The usual m.c. of sound dried fruits is about 12–15%.) Brown (1938) reported that the m.c. above which mites and moulds became obvious was about 20%. Apricots of m.c. 38% were also infested by *Carpoglyphus*, whereas those containing 20–23% moisture were free of mites and in good condition.

It appears that the dried fruits became susceptible to infestation as a result of a rise in m.c., as in the case of cereal products.

VI. Ecological aspects of control

There is a considerable amount of published information on the control of Tyroglyphids. The methods and the main principles have been summarized elsewhere (Solomon, 1943). Mention need be made here only of points which have been revealed or emphasized by the present work.

It can hardly be emphasized too strongly that the surest and most permanent means of control is to keept the m.c. of stored products below the level necessary for Tyroglyphids to live on them. Owing to the rather high moisture requirements of these mites, the critical moisture level is higher than for most other pests of stored products. Newstead & Duvall (1918) stated that badly infested grain usually contained 14% moisture or more, that there was no serious infestation when the m.c. was below 13%, and that the mites could not live when the m.c. was below 12·4%; their figures for flour were approximately the same. Allowing for slight variations in the materials, and in the methods of determining m.c., later work has amply confirmed the validity of these data (Solomon, 1943). Wheat and its products attain 12·3% m.c. in equilibrium with an atmospheric relative humidity about 60%, at normal temperatures, or 13·1% in equilibrium with R.H. about 65%, or 14% in equilibrium with R.H. about 70%.* The relationship is slightly different according to the type of grain or the type of material, e.g. flour, bran or middlings. The corresponding figures for other materials, such as maize, various seeds, or dried fruits, differ in various degrees from those for wheat, as do the m.c. levels at which mites survive, or become abundant (see Solomon, 1943). An indication of the susceptibility of dried fruits to infestation, in relation to m.c., is given in section V.

Data in earlier sections show that, when the m.c. is high enough to permit the development of mites, the severity of the infestation is correlated with the m.c. level. Hence, it is useful to keep the m.c. as low as possible in all circumstances, and to prevent the appearance of particularly moist areas in which the mites can multiply rapidly. This implies that buildings used as storage places should be as weather-proof and as moisture-proof as possible. Grain, flour, etc. in bags should be raised above floor level on timber, particularly when stored over concrete floors at ground level, since concrete tends to transmit moisture very readily. Another advantage of using dunnage is that the dust and small particles falling from the bags can be more readily swept away, instead of being left to accumulate moisture from a damp floor or from the atmosphere. If this is done, the building up of dense mite populations which can migrate to the general bulk of material will in many cases be prevented.

It has been shown in earlier sections that various predators, particularly *Cheyletus*, are often an important factor in reducing the numbers of Tyroglyphids. *Cheyletus*, however, tends to be ineffective in winter conditions, when *Tyroglyphus* usually

* I am indebted to Mr T. A. Oxley for these figures, which are mean values based on published data and on investigations in this laboratory.

multiplies most rapidly. Hence it is difficult to see how this predator could be used to full effect in biological control, unless some control were kept over the physical conditions, particularly moisture. Nevertheless, the predator is of considerable value in many circumstances, because of its successful control of *Tyroglyphus* under summer conditions. In this connexion, the role of the scavenging mite, *Glycyphagus*, in assisting the maintenance and spreading of *Cheyletus* populations, at times when *Tyroglyphus* is scarce, is of some importance. In some circumstances, it might be worth while introducing *Cheyletus*, perhaps together with *Glycyphagus*, into storage places. Often, of course, these mites are already present. But, if they were introduced immediately after a fumigation, for example, this would prevent the possibility of *Tyroglyphus* being re-introduced to an environment made more favourable than before by the absence of predators.

As mentioned earlier, the concentration of *Tyroglyphus* in the more superficial parts of flour in bags makes it possible to fumigate fairly effectively with materials which cannot penetrate into the deeper parts.

VII. Observations in a granary

In this section, some of the data from observations in a Gloucestershire granary containing bulk wheat are given. This will provide concrete examples of some of the phenomena discussed in section III, and of the methods used.

The granary was a large six-storey brick building with wooden floors. Each floor held Manitoba No. 1 grain, to a depth of about 1–2 m., the height from floor to ceiling being about 3 m. The grain had been put into storage in May 1939. There were numerous windows in all storeys, half of them unglazed and only partially covered by loose sacking, to allow ventilation.

Approximately 380 samples of grain were taken from this granary over the period June 1940–Aug. 1941. Each sample consisted of 30–40 c.c. of grain. They were not taken at random or evenly throughout the bulk; a limited number of infested sites were selected, and each one studied by means of samples which were usually taken, by means of a spear, at points in a vertical plane passing through the infested portion of grain. Most of the infested sites were adjacent to one or other of the walls, and the sampling plane usually ran perpendicular from the wall into the bulk.

While the m.c. in the centre of each bulk remained about 11·5%, that of the general surface of the grain ranged from under 12% in summer to over 15% in winter. The surface grain was generally found to be infested by *Glycyphagus*, which usually penetrated less than 20 cm. below the surface, and in places by the predatory mite, *Cheyletus*.

Many parts of the brick walls were damp, particularly in the winter and along the south-west side. This was due chiefly to leakage of the vertical drain-pipes against the outsides of walls. The grain in contact with the wettest parts of the walls became caked together and decomposed, forming a crust several cm. thick, adhering to the brickwork. The m.c. of the grain adjacent to the caked material was sometimes over 20%. The moisture gradients from such damp positions into the general bulk were fairly sharp, approaching closely to the m.c. of the bulk about 0·5 m. from the wall, although a slight gradient could usually be detected as far as 1 m. from the wall. In such positions, the grain within 0·5 m. of the wall was often found to be heavily infested by *Tyroglyphus*, usually together with sparser populations of *Glycyphagus* and of *Cheyletus*.

Similar conditions occurred in places along the opposite wall, where, however, the moisture and the infestations were less pronounced.

The mites did not extend throughout the bulk, but occurred in the moister grain—*Glycyphagus* and sometimes *Cheyletus* in the surface layers, *Tyroglyphus*, *Glycyphagus* and *Cheyletus* in the grain near damp parts of the walls. There was usually a clear correlation between the population density and the m.c. of the grain.

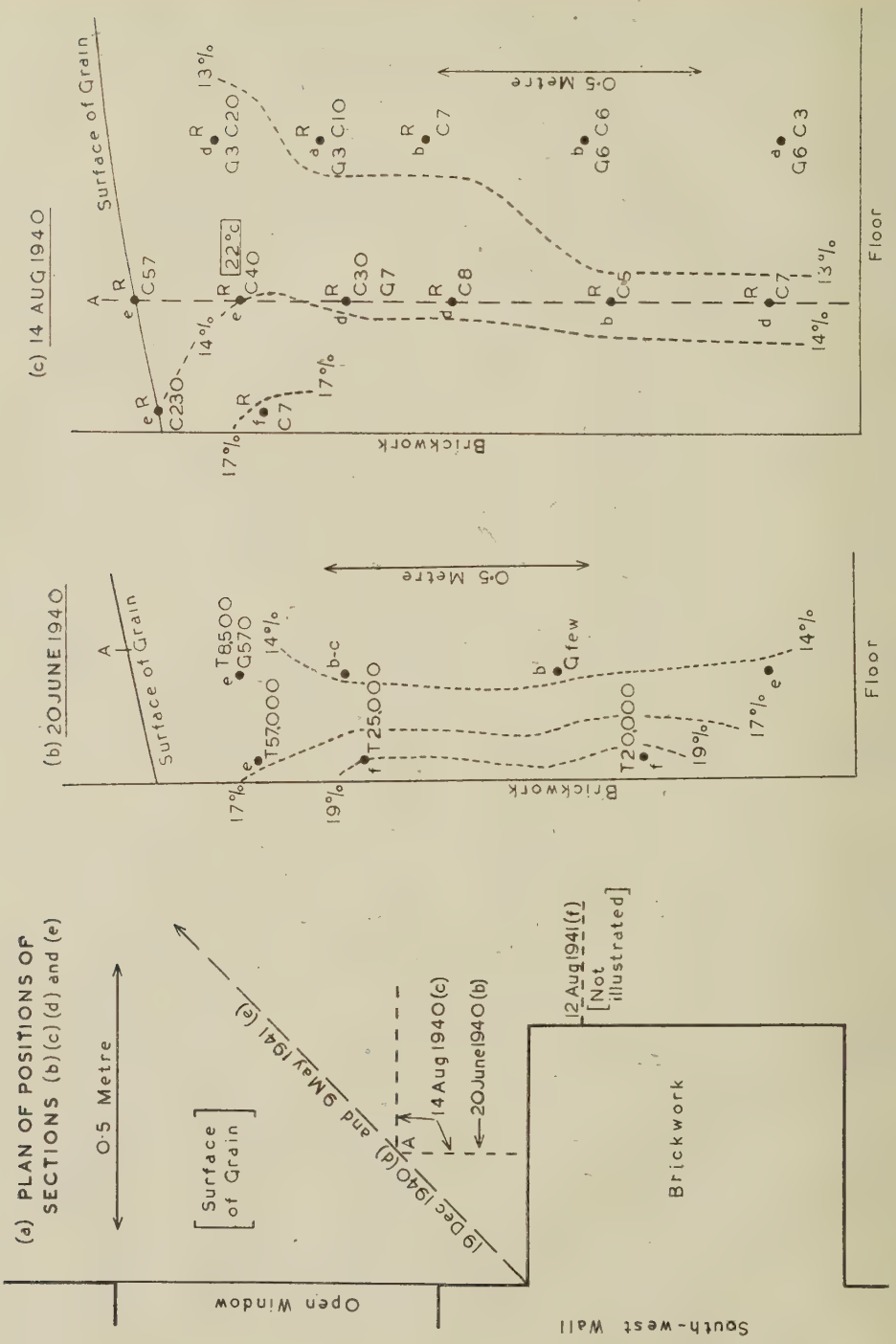
The alternating predominance of *Tyroglyphus* and its predator *Cheyletus* in this granary, and the seasonal nature of the cycle, has been touched upon in section III. Table 3 shows the operation of this cycle in various parts of the granary.

A more detailed study of the matter may be made by reference to Fig. 4, which shows the state of the populations at intervals in one of the sampling positions:

Stage T. Fig. 4*b* shows a dense population of *Tyroglyphus* in the moist grain near a damp wall, in June 1940. No *Cheyletus* were found, but perhaps insufficient samples were taken.

Stage C. In August (Fig. 4*c*), the moisture and temperature conditions were about the same, but *Cheyletus* was found in all samples while *Tyroglyphus* was represented only by remains of dead mites. There seemed to be two possible reasons for this change; either that *Cheyletus* had arrived in this grain since June, or that *Tyroglyphus* had been forced, by the dwindling supply of wheat germ, to migrate into grain that was sufficiently dry to enable *Cheyletus* to control it.

Stage TC. By December (Fig. 4*d*) there had been a considerable increase of moisture in the grain near the wall, and the temperature in the granary had dropped to winter level. *Tyroglyphus* had again reached high numbers, but a little further from the wall than before. In the dampest grain, where it had previously concentrated, there were only the remains of dead mites and grains with the embryos completely destroyed. By May 1941 (Fig. 4*e*), the infested grain was probably already warmer than in


(a) PLAN OF POSITIONS OF SECTIONS (b)(c)(d) and (e)
0·5 Metre
Surface of Grain
Open Window
South-west Wall
19 Dec 1940 (d) and 9 May 1941 (e)
14 Aug 1940 (c)
20 June 1940 (b)
12 Aug 1941 (f) [Not illustrated]
Brickwork
(b) 20 JUNE 1940
Surface of Grain
0·5 Metre
Brickwork
Floor
T8,500
G570
T7,000
T25,000
T20,000
G few
14%
17%
19%
(c) 14 AUG 1940
Surface of Grain
0·5 Metre
Brickwork
Floor
C230
C57
C40
C30
C7
C8
C5
C7
G3 C20
G3 C10
G6 C6
G6 C3
22°c
13%
14%
17%

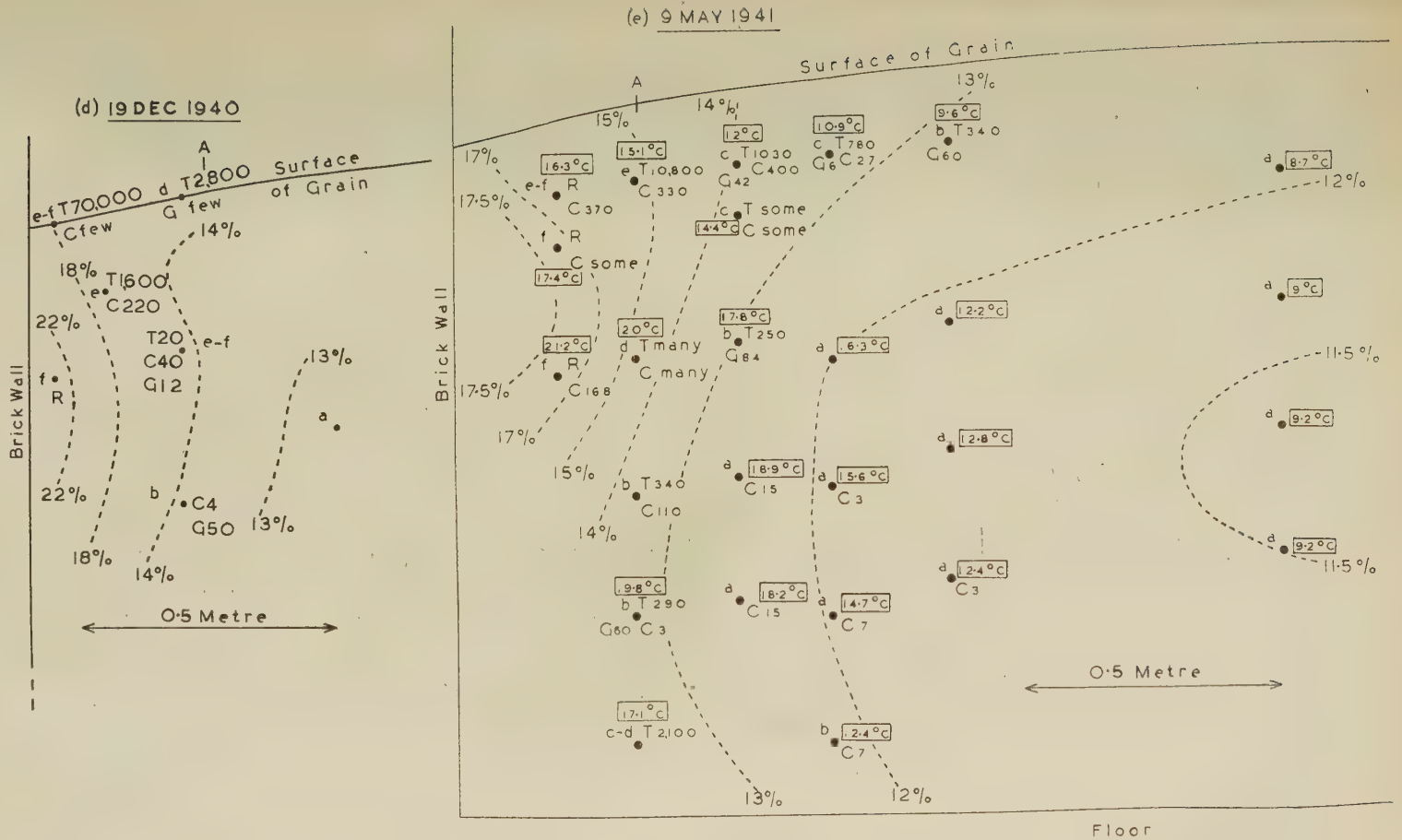


Fig. 4. Data from successive examinations of infested bulk grain, in approximately the same position in a Gloucestershire granary. Fig. (*a*) is a horizontal plan showing the positions of the vertical planes in which samples were taken. In figs. (*b*)–(*e*), the data refer to the sampling points (black dots) around which they are grouped.

Symbols: T 200 etc. = nos. of *Tyroglyphus farinae* (L.) per 100 c.c. grain. G 200 etc. = nos. of *Glycyphagus destructor* (Schr.) per 100 c.c. grain. C 200 etc. = nos. of *Cheyletus eruditus* (Schr.) per 100 c.c. grain. *R* indicates presence of remains of dead *Tyroglyphus*. *a*, *b*, ..., *f* = degree of damage to grain by mites (see p. 85). ------ = lines of equal grain-moisture content. Temperature data in rectangles.

TABLE 3. *Successive changes, during 15 months, in the populations of* Tyroglyphus *in bulk grain in different parts of a Gloucestershire granary*

Symbols *T* and *C* indicate the presence of well-developed populations of *Tyroglyphus* and the predator *Cheyletus*, respectively; *t* indicates the presence of a few living *Tyroglyphus*. The vertical broken lines segregate the *Tyroglyphus*-dominant and *Cheyletus*-dominant populations into separate groups. The seasonal nature of the changes is indicated at the bottom of the table.

	1940							1941							
Position in granary	20 June	5 July	15 Aug.	Sept.	Oct.	8 Nov.	19 Dec.	Jan.	Feb.	Mar.	Apr.	9 May	June	July	11 Aug.
6*a*	TC		*C*			*C*						*TC*			*C*
6*c*			*tC*				*C*								
5*a*							*TC*								
3*a* (Fig. 4)	*T*		*C*				*TC*					*TC*			*tC*
3*b*			*TC*			*C*									
3*c*			*T* (*C*?)			*C*									
3*d*							*TC*					*TC*			
2*a*		*tC*	*tC*			*TC*	*TC*					*TC*			
	[*T* or *TC*] Early summer		[*C* or *tC*] Late summer and autumn					[*TC*] Winter to early summer						[*C* or *tC*] Late summer	

mid-winter. The moisture had changed very little. *Tyroglyphus* had moved further from the wall, into drier grain than before. *Cheyletus* had probably also moved outwards and increased somewhat in numbers.

Stage tC. In August 1941, samples were taken from an adjacent position, indicated in Fig. 4*a*. Remains of *Tyroglyphus* were present in many samples, but only one contained a few living specimens. *Cheyletus* occurred in most of the samples in which the m.c. was above 11·5%. The situation was complicated by the appearance of considerable numbers of a Eupodid mite, *Tydeus* sp., in the moister grain, and there were a few Psocids in some samples. However, the general picture represented a return to the stage found in August 1940. The grain adjacent to the wall was rotting and giving off ammonia; these conditions, as well as a shortage of wheat germ, had probably caused *Tyroglyphus* to move into drier grain where it was unable to survive the onslaught of *Cheyletus*.

A comparison of this cycle of events with those occurring in sampling positions on other floors of the granary revealed a general similarity, as indicated by Table 3.

In the examinations of infested grain, I have had assistance from Mr T. A. Oxley, of this Laboratory. In many cases I am indebted to him for the physical data; in a few cases I have used data from samples which he collected independently and which I examined only in the laboratory.

I am also indebted to Dr J. D. Mounfield, and Dr E. E. Turtle, for assistance mentioned elsewhere.

I am indebted to the Ministry of Food for permission to examine stored materials, and to officers of the Infestation Division of the Ministry who assisted in various ways, particularly Dr J. A. Freeman, Mr F. G. S. Whitfield and Mr F. R. Cann.

Fig. 1*d* is reproduced by courtesy of Messrs S. G. Jary and J. H. Stapley, and the *Journal* of the South-Eastern Agricultural College, Wye, Kent, and Figs. 1*a*, *c*, *f*, *g* and *i* by courtesy of the Royal Society; acknowledgement is also made to the other authors and publications mentioned in the legend.

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The toxicity of certain aliphatic chlorinated hydrocarbons to *Calandra granaria* L. and other insects infesting grain

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(With 1 Text-figure)

An account is given of the biological data obtained during an investigation into the possibility of using certain aliphatic chlorinated hydrocarbons for fumigation of empty grain sacks infested by insects.

The relative toxicity of three mixtures of chlorinated hydrocarbons to adult *Calandra granaria*, was ascertained: the best contained 3 parts ethylene dichloride and 1 part tetrachlorethylene by volume. Among seven species of insects likely to occur in empty sacks, the resistance of *C. granaria* adults to the 3 : 1 mixture was exceeded only by that of *Trogoderma granarium* larvae. The toxicity of the mixture to *C. granaria* was determined over a range of concentrations and periods of exposure: at 20° C. and 70 % R.H. a *concentration* × *time* product of about 1500 mg.-hr./l. was required for 100 % kill. These results were confirmed by inserting tubes containing weevils into bundles of sacks, which were then exposed to fumigant in a steel bin.

The toxicity to adult *C. granaria* of mixtures containing different proportions of ethylene dichloride and tetrachlorethylene increased as the proportion of ethylene dichloride increased. The toxicity of tetrachlorethylene to the weevils was very low in relation to that of ethylene dichloride.

The *concentration* × *time* product for 100 % kill of *C. granaria* at 20° C. by ethylene dichloride alone was about 1100 mg.-hr./l. The products for 50 % kill of *C. granaria* at 10, 15, 20 and 25° C. were in the proportion of 3·0 : 2·0 : 1·0 : 0·7 respectively.

Weevils which recovered from severe narcosis by the 3 : 1 mixture were able to lay viable eggs.

Introduction

Grain is frequently transported and stored in sacks, especially when relatively small quantities are involved. In the absence of effective sterilization, it is necessary to ensure that sacks are not used which may previously have carried grain infested by insects, for such insects may maintain themselves in small numbers for some time on the grains and grain debris remaining in the corners and seams. An attempt has therefore been made to find a method for the sterilization of small numbers of empty sacks, particularly those used on farms for bagging home-grown wheat. Important requirements of the method were that it should be cheap, simple, and reasonably safe in unskilled hands. The possibility has therefore been investigated of fumigation with certain aliphatic chlorohydrocarbons, the use of which has been recommended for some years in the U.S.A. for the large-scale treatment of silo bins, etc. Although these fumigants are not so toxic to human beings as to necessitate rigorous sealing of the fumigation bin or chamber, there still remains the need for reasonable care in avoiding inhalation of the fumigant vapours.

The problem was tackled jointly by members of the Chemistry and Insecticide Sections of the Laboratory. Whilst the chemists were investigating the penetration of the fumigants into bundles of sacks stacked in various ways, the estimation and maintenance of gas concentrations, and methods of airing, the author was responsible for investigating the toxicity of different fumigant mixtures over a range of temperatures to various species and stages of the commoner insects which were likely to be found in empty grain sacks. The data from these two lines of work were combined to provide an estimate of the dose of fumigant which must be applied outside the bundles to achieve and maintain the proper concentration inside them during the selected period of exposure. The estimate was then checked by determination (*a*) of the concentrations of fumigant around tubes of test insects inserted into bundles of sacks, and (*b*) of the kill attained. The final recommendations for this type of fumigation in practice will be published separately.

Fumigants

A survey of data concerning the toxicity to insects of aliphatic hydrocarbons and consideration of the question of availability led to the choice of three of the lower members of the group,

(1) Ethylene dichloride:
(1, 2-dichlorethane, $C_2H_4Cl_2$).
(2) Tetrachlorethylene:
(perchlorethylene, C_2Cl_4).
(3) Carbon tetrachloride:
(tetrachlormethane, CCl_4).

With ethylene dichloride there is a slight risk of inflammability at concentrations in air at ordinary temperatures between 6·2% and about 12·4% by volume (Coward & Jones, 1938), whereas tetrachlorethylene and carbon tetrachloride are both fire-retardent. At the start of the investigation, therefore, these fumigants were used as mixtures of the following compositions, the proportions being parts by volume:

(A) 3 parts $C_2H_4Cl_2$ + 1 part CCl_4.
(B) 3 parts $C_2H_4Cl_2$ + 1 part C_2Cl_4.
(C) 2 parts C_2Cl_4 + 1 part CCl_4.

Experimental technique

For the determination of the comparative toxicities of the various fumigants to the selected species of insects, the Page-Gough apparatus described in general terms by Page & Lubatti (1940) was employed. Batches of about 50 or 100 insects were placed, without food, in cylindrical glass tubes, 2½ in. in length and 1 in. in diameter, closed at both ends by a double layer of muslin. One tube was put in each insect chamber of the apparatus and could be removed at any time without disturbing the exposure of the remainder or appreciably altering the concentration of fumigant in the system. All experiments were carried out at temperatures controlled to ±0·1° C. and at a relative humidity of 70%. Although the amount of fumigant introduced was calculated on the basis of the volume of the apparatus, gas samples were withdrawn into evacuated flasks at the beginning and end of each experiment and the actual concentrations attained were determined by Mr S. E. Lewis of the Chemistry Section by Winteringham's (1942) methods. In most tests the concentrations were based on the determination of the total chlorine in the samples, but in one instance (Table 3, Exp. 2) the constituents of the mixture were determined separately. The composition of the gas in these samples differed little from that of the liquid fumigant applied.

When experiments were being conducted on the penetration of vapour into bundles of sacks exposed to fumigant in a rectangular steel bin, the opportunity was taken of placing test insects in muslin-capped glass tubes within the bundles. Toxicity data were thus obtained under conditions resembling those of practice.

Insects

Most of the work was done with adults of the granary weevil, *Calandra granaria* L., bred on N. Manitoba No. 1 wheat under controlled conditions at 25° C. and 70% R.H. Newly emerged beetles were collected by sieving the cultures at weekly intervals and transferred to fresh grain. They were kept at 25° C. and 70% R.H. for 2 weeks, to allow them to mature sexually, and were subsequently maintained for

1 week at the temperature at which they were to be fumigated. At the time of test the weevils were therefore 3–4 weeks old. Beetles in excess of the number required were randomly mixed and the desired number of batches counted into the insect tubes on the day before fumigation, thus ensuring time for recovery from the disturbance inherent in the method of counting with a suction tube (Parkin & Green, 1943). Other species of insects, although not of known age, were also bred at 25° C. and 70% R.H. and conditioned to the temperature of fumigation for at least 1 week. One or more batches of insects, handled in the same manner as those fumigated, except for exposure to the fumigant, were kept as controls in each experiment.

After fumigation, the insects were transferred to 3 × 1 in. glass tubes containing a small amount of suitable food and closed with a perforated cork covered with muslin. During the observation period, the insects were kept at 25° C. and 70% R.H. The numbers alive, i.e. showing any form of spontaneous movement, were counted after 5, 10 and 15 days, but, in the later experiments, the count on the 15th day was omitted, as experience showed that the occasional slight differences from the 10th day count were insufficient to warrant the labour of an additional examination.

Experimental results

Comparative toxicity of the three mixtures

The comparative toxicity to adult *C. granaria* of the three mixtures of chlorinated hydrocarbons was determined at 20° C. (see Table 1).

Table 1. *Comparative toxicity to adult* C. granaria *of three mixtures of chlorinated hydrocarbons*

20 hr. exposure at 20° C. and 70% R.H.

Fumigant	Conc. (mg./l.)	No. of insects	Kill (%)
$3C_2H_4Cl_2 : 1CCl_4$	41·5	794	12·7
$3C_2H_4Cl_2 : 1C_2Cl_4$	38·0	800	68·8
$2C_2Cl_4 : 1CCl_4$	40·7	790	4·1

The mixture of ethylene dichloride and tetrachlorethylene proved outstandingly more toxic than the others and subsequent work was confined to these two substances.

Resistance of different species of insects

This series of experiments was carried out to select a reasonably resistant species for further work, rather than to determine accurately the relative resistance of the commoner insects likely to be found in empty sacks. The fumigations were done with the 3 : 1 mixture of ethylene dichloride and tetrachlorethylene and the results are shown in Table 2.

Adult *C. granaria* were chosen as the test insects for subsequent work, because of their relatively high resistance to the chlorinated hydrocarbon mixture, and because they were much easier to rear and handle than *Trogoderma* larvae.

The toxicity to C. granaria *of 3 : 1 ethylene dichloride and tetrachlorethylene*

Having determined the best of the three fumigant mixtures and a suitable test insect, more detailed work was done to establish the relationship between concentration of the fumigant, period of exposure, and mortality of the weevils at 20° C. and 70% R.H. The data obtained are shown graphically in Fig. 1, in which each percentage kill value was based on the exposure of, normally 200, but occasionally up to 800, weevils. Although the insects were bred under carefully standardized conditions, the dispersion of the points indicates the considerable variation in resistance encountered during the several months

Table 2. *Resistance of various species of insects to a* 3 : 1 *mixture of* $C_2H_4Cl_2$ *and* C_2Cl_4

20 hr. exposure at 20° C. and 70% R.H.

Species	Stage	Conc. (mg./l.)	No. of insects	Kill (%)
Trogoderma granarium	Larva	31·6	151	11·3
Calandra granaria	Adult	28·8	401	34·7
Tribolium castaneum	Adult	30·0	686	94·1
Tribolium castaneum	Larva	29·6	200	96·0
Tribolium confusum	Adult	26·9	503	100
Tribolium confusum	Larva	23·9	202	100
Ptinus tectus	Adult	30·4	240	100
Ptinus tectus	Larva	24·3	100	99·0
Ephestia kuehniella	Larva	34·2	200	100
Plodia interpunctella	Larva	23·9	99	100

occupied by these tests. In subsequent experimental work it was assumed that 50% kill of the weevils could be secured by a *concentration* × *time* product of about 600–700 mg.-hr./l., and 100% kill by a $c \times t$ product of about 1500 mg.-hr./l.

Experiments with test insects in sacks

As soon as it was apparent that a $c \times t$ product of about 1500 mg.-hr./l. (i.e. 31 mg./l. for 48 hr.) was required for 100% kill of *C. granaria* weevils by the mixture of 3 parts ethylene dichloride with 1 part tetrachlorethylene, experiments were carried out to ascertain whether test insects introduced into bundles of sacks could be killed by this dosage under conditions simulating those of practice. These experiments were made in collaboration with the Chemistry Section, who had simultaneously been working on the penetration of fumigants into, and the rate of airing of the gases from, bundles of sacks by the method of fumigation described below.

Four bundles, each of twenty sacks rolled together

and tied with string, were stood in an upright position in two tiers of two in a rectangular steel bin, 2 ft. × 2 ft. 3 in. in cross-section and 5 ft. 6 in. in height and provided with a tightly fitting lid. Tubes, each containing 100 weevils, were inserted one-third of the length from the top and bottom of each bundle between the tenth and eleventh sacks: other tubes were exposed in the free space above the sacks. The added and the bin closed. Gas samples were withdrawn at intervals during the 48 hr. exposure period through previously inserted lead capillary tubing. The results are summarized in Table 3.

These data showed that a lethal concentration of the mixture could be built up and maintained within bundles of sacks rolled and tied in the manner described, and confirmed that a dose of about 31 mg./l.

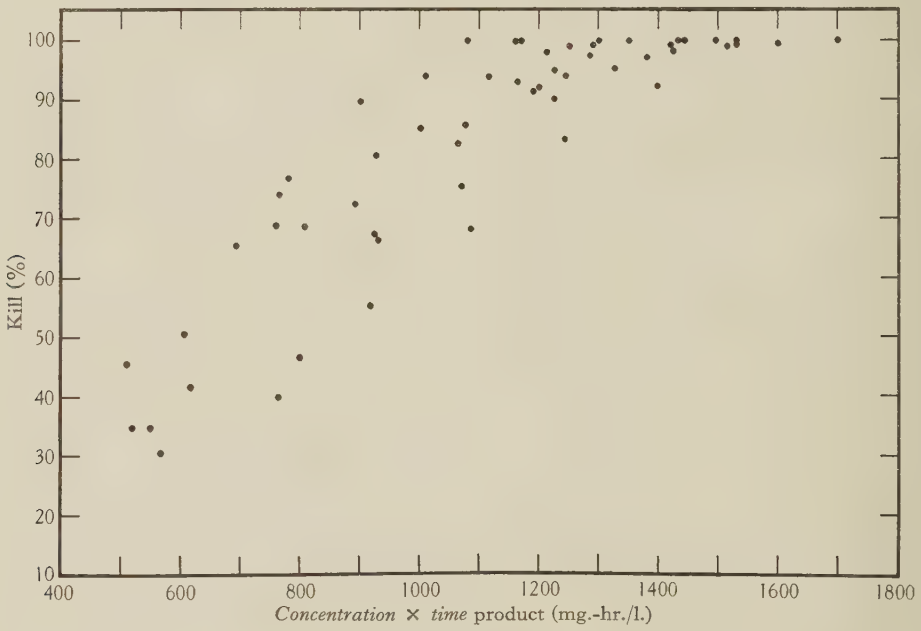

Fig. 1. The toxicity to adult *Calandra granaria* of a 3 : 1 mixture of ethylene dichloride and tetrachlorethylene at 20° C. and 70 % R.H.

TABLE 3. *Data from experiments with* C. granaria *in bundles of sacks and a* 3 : 1 *mixture of* $C_2H_4Cl_2$ *and* C_2Cl_4

48 hr. exposure at 19–20° C.

Exp. no.	Fumigant (ml.)	Position of insects	Mean conc. (mg./l.)	No. of insects	Kill (%)
1	500	Free space	20	200	78·5
		Upper bundles	10	400	13·0
		Lower bundles	< 5	600	2·7
2	466 + 466	Free space	38	300	100
		Lower bundles	29·5	400	99·8
	Controls	—	—	293	5·8

insects were 3–4 weeks old and had been maintained for 7 days at the temperature of the test (19–20° C.). In the first experiment the fumigant mixture was sprayed with a hand syringe over the top of the bundles and the lid quickly clamped in position. In the second experiment one-half of the liquid was sprayed on the lower bundles before the upper bundles were inserted and the remaining fumigant is required to be held for 48 hr. in order to achieve 100 % kill of adult *C. granaria* at 20° C.

The toxicity to C. granaria *of mixtures containing different proportions of ethylene dichloride and tetrachlorethylene*

As it was not known whether the 3 : 1 mixture of ethylene dichloride and tetrachlorethylene was the

most toxic combination, tests were undertaken in the Page-Gough apparatus with the two components separately and in mixtures representing three different proportions by volume. The doses were calculated to give in all instances a concentration of 30 mg./l. During the tests, which occupied five consecutive days, weevils were drawn at random from a general population of about 2500 and were exposed for 20 hr. at 20° C. and 70% R.H. The results are given in Table 4.

TABLE 4. *Toxicity to adult* C. granaria *of different mixtures of ethylene dichloride and tetrachlorethylene*

20 hr. exposure at 20° C. and 70 % R.H.

Mixture (parts by vol.) $C_2H_4Cl_2$	Mixture (parts by vol.) C_2Cl_4	Conc. (mg./l.)	No. of insects	Kill (%)
100	0	29·2	401	65·3
75	25	28·8	401	34·7
50	50	29·2	384	42·6
25	75	26·5	400	8·0
0	100	20·3	399	1·5
Controls		—	250	0·4

TABLE 5. *Information from literature on toxicities of* $C_2H_4Cl_2$ *and* C_2Cl_4

Insect (adult stage)	Temp. (° C.)	Exposure period (hr.)	Conc. for 50 % kill $C_2H_4Cl_2$ (mg./l.)	Conc. for 50 % kill C_2Cl_4 (mg./l.)	Author
C. granaria	25	5	138	—	(1)
	?	5	—	91·2	(2)
	20	5	—	101	(3)
C. oryzae	25	5	35	—	(4)
	25	5	31	—	(1)
	20	5	—	90	(3)
T. confusum	25	5	33	—	(5)
	25	5	46	54	(6)
	25	5	35	—	(4)
	25	5	37·5	—	(1)

(1) Shepard *et al.* (1937).
(2) Ferguson (1936).
(3) Busvine (1942).
(4) Shepard & Lindgren (1934).
(5) Strand (1930).
(6) Fisk & Shepard (1938).

Ethylene dichloride was more toxic by itself than in any mixture with tetrachlorethylene, which, when used alone under the conditions of this experiment, had virtually no lethal effect. A particularly sharp fall in toxicity was observed when the proportion of ethylene dichloride in the mixture became less than 50%. The concentration of the 25 : 75 mixture in the apparatus was lower than expected and that of the tetrachlorethylene alone was relatively very low, indicating the probability of adsorption of tetrachlorethylene somewhere in the apparatus.

Comparison of the data in Tables 1 and 4 indicates that there is probably antagonism between ethylene dichloride and carbon tetrachloride when applied as a 3 : 1 mixture, whereas the toxicity of the 3 : 1 mixture of ethylene dichloride and tetrachlorethylene is approximately that expected of the former component alone.

Information in the literature upon which a comparison may be made of the toxicities of ethylene dichloride and tetrachlorethylene is scanty and is summarized in Table 5.

Only the figures for *C. oryzae* show any marked difference in the toxicities of the two fumigants, but a part of this difference will be due to the effect of temperature. The data for *C. granaria* indicated that tetrachlorethylene was more toxic than ethylene dichloride. Additional tests were therefore undertaken to confirm the data in Table 4. Table 6 summarizes the results.

TABLE 6. *Confirmatory experiments upon the toxicity to adult* C. granaria *of* $C_2H_4Cl_2$ *and* C_2Cl_4

20 hr. exposure at 20° C. and 70 % R.H.

Fumigant	Conc. (mg./l.)	No. of insects	Kill (%)
$C_2H_4Cl_2$	31·6	800	44·9
	29·6	806	48·6
	30·9	798	69·9
	28·3	786	68·2
Controls	—	600	0·3
C_2Cl_4	18·4	802	4·4
	22·0	802	3·3
	28·8*	799	11·3
	28·8*	803	6·0
Controls	—	501	0·6
$3C_2H_4Cl_2 : 1C_2Cl_4$	28·4	600	30·6
Control	—	100	1·0

* Dose increased to give approximately 30 mg./l. final concentration.

These results completely confirm the conclusions drawn from the data of Table 4. Even when the dose of tetrachlorethylene was adjusted to allow for adsorption in the apparatus and thus to give a concentration during fumigation of approximately 30 mg./l., the kill achieved was very low compared with that caused by ethylene dichloride. The disparity between the mortalities obtained with ethylene dichloride was no doubt related to the fact that the two pairs of experiments were done with an interval of 6 weeks and again illustrates the variation in resistance which may be encountered in stocks of weevils, although bred under controlled conditions.

Most of the data in Table 5 refer to an exposure period of 5 hr. at a temperature of 25° C. In case high concentrations of tetrachlorethylene applied for short periods of exposure should prove more toxic than lower concentrations applied for correspondingly longer periods, a comparison of the toxicities

of ethylene dichloride and tetrachlorethylene was made with exposures of 4–7 hr. at 25° C. and 70% R.H. (see Table 7).

TABLE 7. *Toxicity to adult* C. granaria *of high concentrations of* $C_2H_4Cl_2$ *and* C_2Cl_4 *applied for short periods of exposure*

4–7 hr. exposure at 25° C. and 70% R.H.

Fumigant	Conc. (mg./l.)	Exposure (hr.)	No. of insects	Kill (%)
$C_2H_4Cl_2$	94·8	4	203	51·7
		5	200	69·5
		6	202	81·2
		7	205	94·6
C_2Cl_4	95·4	4	200	7·0
		5	191	4·1
		6	206	16·0
		7	203	44·3
Controls	—	7	200	1·5

TABLE 8. Concentration × time *products for a* 50% *kill of adult* C. granaria *at different temperatures, using a dosage of* 30 *mg./l. of ethylene dichloride*

Temp. (° C.)	Approx. $c \times t$ (mg.-hr./l.)	Ratio to $c \times t$ at 20° C.
10	1470	3·0
15	965	2·0
20	490	1·0
25	340	1·0

With high concentrations and short periods of exposure at 25° C., tetrachlorethylene was still markedly less toxic than ethylene dichloride.

The data obtained in this section of the work led to a reconsideration of the danger of inflammability connected with the use of ethylene dichloride alone. A risk arises only with concentrations between 6·2% and about 12·4% by volume in air. Since the lower limit, corresponding to 255 mg./l. at 20° C., is well above the level of concentration likely to be recommended for use in bin fumigations of empty sacks, it was decided to continue the investigation with ethylene dichloride alone.

The toxicity of ethylene dichloride to C. granaria *at different temperatures*

Beetles, maintained for 1 week previously at the temperature of fumigation, were exposed to doses of approximately 30 mg./l. of ethylene dichloride for appropriate periods of exposure at 10, 15, 20 and 25° C. respectively. At each temperature, the $c \times t$ products employed were plotted against the observed percentage mortalities and the product corresponding to a 50% kill ascertained approximately by interpolation: these products are given in Table 8.

If, for practical purposes, the $c \times t$ product for a given level of kill and temperature be assumed to be constant, the ratios express the relation between the concentrations which must be maintained at different temperatures in order to achieve the same kill in a set period of exposure. For example, to produce a 50% kill of the weevils, about three times the concentration effective at 20° C. will be required at 10° C. A similar relationship holds for the relation between period of exposure and temperature for a given concentration.

The curve representing $c \times t$ at 20° C. plotted against percentage mortality indicated that a product of about 1100 mg.-hr./l. should be adequate for 100% kill of adult *C. granaria*: this compares with the product of 1500 mg.-hr./l. for the 3 : 1 mixture of ethylene dichloride and tetrachlorethylene. Shepard *et al.* (1937) give the only comparable information in the literature upon the dosage of ethylene dichloride required for 100% kill of *C. granaria*: they found that 256 mg./l. for 5 hr. ($c \times t = 1230$ mg.-hr./l.) were necessary at 25° C.

In practice, a fumigation period of 48 hr. or more would normally be used and a minimum average concentration of 23 mg./l. of ethylene dichloride would be required in the sacks. This is far below the concentration of 255 mg./l. at 20° C. at which the risk of inflammability starts. Provided that the proper general precautions are taken in handling the fumigant and carrying out the fumigation, the only time when there might be a local concentration of vapour high enough to involve a risk of inflammability would be whilst the liquid is being measured and applied. Naked lights should not be permitted in the vicinity during this period. Smoking should also be prohibited when aliphatic chlorinated hydrocarbons may be present in the atmosphere, because of the production of phosgene on thermal decomposition of this group of compounds (Jacobs, 1941).

Observations on the effects of aliphatic chlorohydrocarbons upon C. granaria

All beetles were narcotized and inactive on removal from the fumigant atmosphere, unless the final kill proved to be extremely low. The majority of weevils which recovered did so during the first 5 days of the observation period, relatively few recovering between the 5th and 10th days. Beetles which recovered appeared always to regain full activity and fed upon the grain in the observation tubes.

After an experiment in which 95 beetles out of 100 were killed with a 3 : 1 mixture of ethylene dichloride and tetrachlorethylene, the five insects alive on the 10th day after fumigation were placed on clean grain and maintained at 25° C. and 70% R.H. Ten weeks later, eighty weevils were present, showing that severe narcosis with these chlorinated hydrocarbons had caused no appreciable injury to the reproductive organs of the females.

This work has formed part of the programme of research of the Pest Infestation Laboratory, and this account is published with the permission of the Department of Scientific and Industrial Research.

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Toxicity to rainbow trout and minnows of some substances known to be present in waste water discharged to rivers

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(With 4 Text-figures)

Toxicity to fish (rainbow trout or minnows) of solutions of several pure substances has been measured under controlled conditions. The substances (sodium arsenite, sodium arsenate, sodium picrate, sodium dinitrophenate, zinc sulphate, potassium chromate, potassium dichromate, ammonium chloride, and ammonium sulphate) were dissolved in distilled water, in Watford tap water, or in mixtures of distilled water and tap water.

INTRODUCTION

There is a fairly extensive literature on the toxicity to fish of solutions of poisonous substances known to be present in many kinds of industrial waste waters. Much of the work in this field has been carried out in America, where tests of toxicity have been made on fish which are not found in the British Isles. The literature on the toxicity to British fish is not extensive and in many cases the conditions under which tests have been made have not been stated.

The work described in the present paper was carried out during investigations of methods of treatment and disposal of industrial waste waters. The object of treatment was to reduce the concentration of toxic substances to a point at which, when discharged to a river of known size, they would not cause undue pollution or damage to fisheries. For this purpose the tests were made to ascertain the maximum concentration of toxic substances which do not affect fish during periods of a few days. It is probable that smaller concentrations acting over long periods in a river would damage a fishery, either by their continued action on the fish themselves or by their toxic action on other aquatic organisms which serve as the food of the fish. Nevertheless, determinations of the threshold toxic concentration during periods of a few days are valuable as a guide to the relative toxicity of the constituents to be removed from the industrial wastes.

Several independently variable factors influence the toxicity to fish of solutions of a given substance; they include the type of water in which the substance

is dissolved, the concentration of the poison, the temperature, *p*H value, and degree of oxygenation of the solution, and the species, size, and condition of the fish used. Unless the conditions under which tests are made are controlled, the results obtained may vary within wide limits.

The greatest source of error in making tests of toxicity is probably the difference in susceptibility of individual fish to a given poison; it is largely a matter of chance whether the fish taken from the stock tank for any particular test are a representative sample of the stock as a whole.

Methods

During the investigations on which this paper is based, attempts have been made to improve the technique of making the tests. In the earlier work, the temperature at which tests were made was not standardized, but was maintained at as uniform a value as possible during each test; latterly the temperature of all test solutions has been controlled at 18° C.

Some of the substances used were dissolved in Watford tap water (temporary hardness 26·5 parts $CaCO_3$ per 100,000; total hardness 27·8 parts $CaCO_3$ per 100,000; *p*H value about 7·4), others were dissolved in distilled water, which had been aerated for several hours. Aeration was continued during the tests. In some instances the concentration of dissolved oxygen in the test solution was determined by the Winkler method at the beginning and end of each experiment. Substances such as chromates interfere with the Winkler test; experiments were made, therefore, to determine the rate at which air should be bubbled through 30 l. of water containing ten yearling trout to maintain the concentration of dissolved oxygen at a satisfactory value; in subsequent tests, air was supplied at the necessary rate to all test solutions. When a test was continued for several days, the test solution was renewed at intervals of 2 days.

Minnows (*Phoxinus phoxinus* L.) and rainbow trout (*Salmo gairdneri* Richardson var. *shasta*) were used as test animals; the age of the minnows was not known; the trout were about 1 year old and 3–4 in. long.

The fish required for the experiments were kept without difficulty in large glass tanks through which was passed a slow stream of tap water, which had first been passed through a tower containing activated carbon to destroy any free chlorine. The water was aerated by blowing compressed air through sintered glass diffusers near the bottom of the tanks. The fish were fed once a day on minced raw bullock's heart.

Water to be used in any particular test was aerated overnight; early on the following morning a weighed amount of the test substance, or a measured volume of a concentrated solution, was added and the volume, temperature, and if necessary the *p*H value of the solution were adjusted to predetermined values. Fish for the test were caught from the stock tank in a large net; by so doing a fairly representative sample of fish was obtained.

Fish were removed individually from the test solution and were transferred to fresh water when they lost equilibrium; in this condition they usually lay on their side on the bottom of the tank, or floated sideways on the surface of the water, or drifted helplessly with the current. It was noted whether the fish transferred to fresh water recovered or died.

Toxicity of a solution to individual fish was expressed as 100 times the reciprocal of the period (in minutes) during which each fish was immersed; the arithmetic mean of individual toxicities of all fish immersed in any given solution has been called the average toxicity. The mean period of immersion of fish was taken as 100 times the reciprocal of the average toxicity and is expressed in minutes. To supplement the average values of toxicity, which give no indication of the spread of the results, the standard deviation from the average toxicity has been quoted in the tables. Standard deviations have been calculated from the formula

$$\sigma = \sqrt{\frac{\Sigma\,(t - t_a)^2}{n - 1}},$$

where σ is the standard deviation,

t is the toxicity to individual fish,

t_a is the average toxicity,

and n is the number of fish immersed.

Results of the tests

Sodium arsenite ($NaAsO_2$) *and sodium arsenate* (Na_2HAsO_4)

Measured amounts of a concentrated solution of the two substances were added to Watford tap water and the resulting solution was neutralized by addition of hydrochloric acid; the concentration of arsenic was checked by analysis. All solutions were well aerated before and during immersion of fish; experiments were made to show that solutions of arsenite are not oxidized by aeration. Results of determination of toxicity to minnows given in Table 1 are shown graphically in Fig. 1.

Neutralized solutions of sodium arsenite were more toxic to minnows than solutions of sodium arsenate containing an equal concentration of arsenic. The limiting concentration, below which solutions containing arsenic were non-toxic to minnows, was not determined by direct experiment. In a solution of sodium arsenite containing approximately 20 p.p.m. As, minnows survived for a mean period of about 36 hr. before overturning. In a solution of sodium arsenate containing approxi-

mately 250 p.p.m. As, they survived for about 16 hr. before overturning.

Wiebe (1930) found that sodium arsenite, dissolved in River Mississippi water in a concentration equivalent to 5 p.p.m. As, was not deleterious to several species of fish, including black bass, white crappie, bluegill, golden shiner, and goldfish. Ellis (1937) found that arsenite in a concentration equivalent to 1 p.p.m. As in distilled water killed *Daphnia magna*, and Surber & Meehan (1931) stated that 1·5 p.p.m. As present in arsenite in River Mississippi water were harmless to important fish-food organisms, though concentrations of arsenite equivalent to between 1·9 and 3 p.p.m. As were fatal to chironomid larvae, mayfly nymphs, and freshwater shrimps. For *Daphnia magna*, Anderson (1944) found the threshold concentration for immobilization by sodium arsenate dissolved in Lake Erie water to be 31 p.p.m. Na_2HAsO_4 (14 p.p.m. As).

The point at which minnows, immersed in solutions of sodium arsenite, were judged to have lost equilibrium was well defined; in solutions of sodium arsenate, minnows lost control of balance much more gradually and in some cases it was difficult to decide with certainty at what point they had permanently overturned. None of the fish which had been immersed in solutions of sodium arsenate or sodium arsenite recovered after removal to fresh water.

TABLE 1. *Toxicity to minnows* (Phoxinus phoxinus L.) *of neutralized solutions in Watford tap water*

Test material	Test solution: Concentration	Temperature (° C.) Min.	Temperature (° C.) Max.	Dissolved oxygen (parts per 100,000) Initial	Dissolved oxygen (parts per 100,000) Final	Initial *p*H	Toxicity* Mean	Toxicity* Standard deviation	Mean immersion period (min.) before overturning	No. of fish immersed
	(p.p.m. As)									
Sodium arsenite	953	11·5	17·5	—	—	8·0	1·83	0·71	54·6	12
	669	13·0	13·0	—	—	7·9	1·25	0·29	80·0	12
	478	12·2	17·0	—	—	8·0	1·04	0·45	96·1	12
	384	11·5	13·0	—	—	8·0	0·88	0·28	114·2	12
	290	11·5	17·0	—	—	8·0	0·54	0·19	186	12
	195	12·2	17·0	—	—	8·1	0·40	0·14	248	12
	98	11·5	13·0	—	—	8·0	0·21	0·07	483	12
	47·5	14·3	18·2	—	—	7·8	0·088	0·026	1142	12
	17·8	12·3	—	—	—	7·8	0·046	0·016	2174	12
Sodium arsenate	2970	16·8	19·4	0·86	0·78	8·3	0·488	0·079	205	12
	2370	17·0	22·5	0·88	0·79	8·3	0·403	0·092	248	12
	1780	16·5	20·0	0·86	0·83	8·3	0·340	0·081	294	12
	1210	15·5	19·0	0·91	0·87	8·2	0·226	0·046	442	12
	820	13·0	—	0·89	—	7·9	0·214	0·027	467	6
	610	17·3	21·3	0·84	0·83	8·0	0·184	0·025	543	12
	234	16·1	20·0	0·83	0·83	8·2	0·105	0·022	951	12
	(p.p.m. $C_6H_2(NO_2)_3OH$)									
Sodium picrate	2000	16·7	17·2	0·99	—	7·3	0·521	0·105	192	6
	1500	16·8	16·8	0·92	—	7·4	0·422	0·035	237	6
	1000	14·8	17·9	—	—	7·7	0·271	0·062	369	6
	700	16·4	16·7	1·05	—	7·7	0·211	0·063	474	6
	400	13·0	17·1	—	—	7·9	0·121	0·015	826	6
	309	13·3	16·7	—	—	7·9	0·089	0·021	1124	6
	200	13·0	20·0	0·92	—	7·8	0·064	0·041	1563	6
	(p.p.m. $C_6H_3(NO_2)_2OH$)									
Sodium dinitrophenate	250	17·2	17·6	0·88	—	7·9	5·64	2·01	17·7	6
	200	17·5	17·5	0·83	—	7·8	4·51	0·18	22·2	6
	150	12·4	12·4	0·98	—	7·7	3·35	0·49	29·9	6
	100	16·5	16·5	0·88	—	8·1	1·64	0·26	61·0	6
	70	12·7	—	0·84	—	8·1	0·938	0·15	107	6
	50	15·3	—	0·90	—	8·1	0·478	0·079	209	6

* For explanation, see text.

Sodium picrate and sodium dinitrophenate

Stock solutions of sodium picrate and sodium dinitrophenate were prepared by dissolving picric

acid and dinitrophenol in water containing a slight excess of sodium hydroxide and neutralizing the solution with hydrochloric acid. Measured portions of stock solutions were diluted with Watford tap water to give the test solutions; these were well aerated before and during the immersion of fish. Results of tests with minnows are given in Table 1 and are shown graphically in Fig. 2.

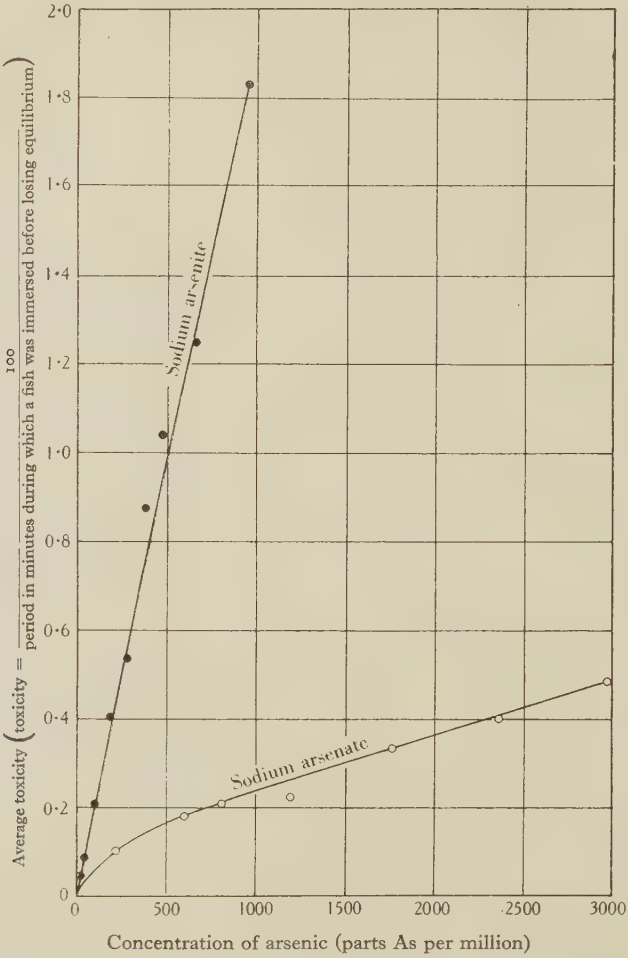

Fig. 1. Toxicity of sodium arsenite ($NaAsO_2$) and sodium arsenate (Na_2HAsO_4) to minnows (*Phoxinus phoxinus* L.)

At a given concentration, dinitrophenol was much more toxic than picric acid. For example, in solutions containing 200 p.p.m. picric acid and dinitrophenol, the mean periods of immersion before overturning were approximately 26 hr. and 22 min. respectively. Extrapolation of the toxicity curves to zero toxicity indicates that the limiting concentration below which neutralized solutions of dinitrophenol are non-toxic to minnows is probably about 30 p.p.m. dinitrophenol; the limiting value for neutralized solutions of picric acid is probably of the same order.

The time at which minnows immersed in solutions of sodium picrate or of sodium dinitrophenate overturned was clearly defined. No fish recovered on removal to fresh water.

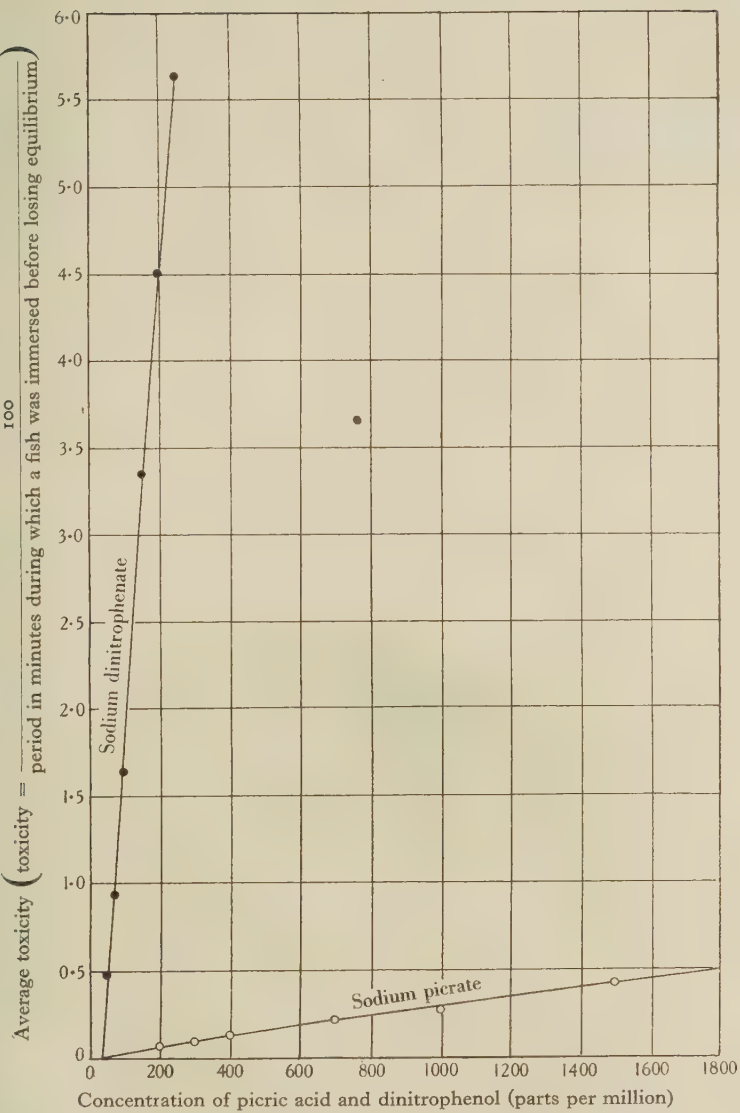

Fig. 2. Toxicity of neutral solutions of sodium picrate and sodium dinitrophenate to minnows (*Phoxinus phoxinus* L.)

Zinc sulphate

A test was made of the toxicity to rainbow trout of only one concentration of zinc sulphate in distilled water. Distilled water was aerated for about 16 hr. before the zinc sulphate was dissolved in it; the temperature of the solution was maintained at 18° C. No adjustment of *p*H value was made. Ten trout were immersed in 30 l. of solution and aeration was continued during the test. The result is given in Table 2. The mean period of immersion of trout before losing equilibrium in a solution containing zinc sulphate in a concentration equivalent to 25 p.p.m. Zn was 133 min. The end-point of the period of immersion was particularly well

defined. All fish died soon after transfer to fresh water.

Potassium chromate and potassium dichromate

In tests of toxicity to rainbow trout of solutions of potassium chromate and potassium dichromate, the substances were dissolved in distilled water which had been aerated for about 16 hr. The temperature of the solutions was maintained at 18° C.; no adjustment of *p*H value was made. Aeration of solutions was continued during tests. Results of the tests are summarized in Table 2 and are shown graphically in Fig. 3. At equivalent concentrations of chromium, solutions of potassium dichromate were more toxic than solutions of potassium chromate. As a result of a review of data on the toxicity of acid wastes, Ellis (1937) came to the conclusion that the toxicity of a solution with a *p*H value less than 5·0 is due at least in part to its acidity; in a solution with a *p*H value greater than 5·0 lethality factors other than concentration of hydrogen ion play a major part. Results of the present investigation confirm this view. The most dilute solutions tested contained potassium chromate and potassium dichromate in concentrations equivalent to 20 p.p.m. Cr. Both solutions had *p*H values greater than 5·0 and had similar toxicities to trout; in the solution of potassium chromate the mean period of immersion before overturning was about 60 hr.; the corresponding period for the solution of potassium dichromate was about 72 hr.

With an increase of concentration the *p*H value of solutions of potassium dichromate fell below 5·0 and these solutions were considerably more toxic than solutions containing an equivalent concentration of potassium chromate. The toxicity of solutions of potassium chromate was almost proportional to the concentration of dissolved substance; the toxicity of solutions of potassium dichromate was not a linear function of concentration. The toxicity increased rapidly with an increase in concentration up to the equivalent of 400 p.p.m. Cr; between 400 and 1000 p.p.m. the toxicity increased only slightly; above 1000 p.p.m. toxicity again increased rapidly

TABLE 2. *Toxicity to yearling rainbow trout* (Salmo gairdneri *Richardson var.* shasta) *in well-aerated distilled water*

Temperature of test solution 18° C.

Test material	Test solution: Concentration		Initial *p*H	Toxicity*: Mean	Toxicity*: Standard deviation	Mean immersion period (min.) before overturning	No. of fish immersed
Zinc sulphate	(p.p.m. Zn) 25		5·7	0·75	0·17	133	10
	(p.p.m. Cr)	(p.p.m. K_2CrO_4)					
Potassium chromate	2000	7470	8·0	2·44	0·90	41·0	10
	1500	5602	8·0	1·90	0·58	52·6	10
	1000	3735	8·0	1·26	0·74	79	20
	800	2988	7·3	1·31	0·53	76	30
	600	2241	7·8	0·76	0·15	131	10
	500	1867	7·0	0·58	0·16	172	10
	400	1494	7·8	0·57	0·29	175	10
	300	1120	7·4	0·37	0·15	272	10
	200	747	7·3	0·27	0·11	374	10
	50	187	6·5	0·050	0·066	2000	10
	20	75	6·6	0·028	0·010	3580	10
	(p.p.m. Cr)	(p.p.m. $K_2Cr_2O_7$)					
Potassium dichromate	2000	5658	4·1	4·20	1·59	23·8	20
	1500	3244	4·2	2·53	1·25	39·5	10
	1000	2829	4·1	1·83	0·73	54·6	10
	800	2263	4·2	1·74	0·53	57·5	10
	600	1697	4·4	1·79	1·41	55·9	30
	500	1415	4·3	1·65	0·80	60·6	30
	400	1132	4·4	1·61	0·87	62·1	30
	300	849	4·6	1·19	0·68	84·0	20
	250	707	4·7	0·67	0·52	148	10
	200	566	5·4	0·53	0·60	188	10
	50	142	5·0	0·051	0·044	1946	10
	20	57	5·5	0·023	0·008	4342	10

* For explanation, see text.

with concentration. The limiting concentration below which solutions of potassium chromate and potassium dichromate were non-toxic to rainbow trout appeared to be equivalent to slightly less than 20 p.p.m. chromium.

Ellis (1937) found that chromic acid in hard water, in a concentration of 100 p.p.m. CrO_3 (52 p.p.m. Cr) did not kill goldfish in 100 hr.; in distilled water, the same concentration of chromic acid killed goldfish in 30–35 min. F. T. K. Pentelow (private communication) showed that potassium dichromate dissolved in Nottingham tap water was slightly toxic to brown trout (*Salmo trutta* L.) in a concentration equivalent to 5·2 p.p.m. Cr. At this concentration one fish overturned after immersion for 26¼ hr.; three other fish were apparently unharmed after immersion for 48 hr. Anderson (1944) found the threshold concentration for the immobilization of *Daphnia magna* by chromic acid dissolved in Lake Erie water to be less than 0·3 p.p.m. Cr.

There was little difficulty in deciding the point at which trout immersed in solutions of potassium chromate or of potassium dichromate lost equilibrium.

Fish which had been immersed in solutions containing the highest concentration of chromate died soon after transfer to fresh water; some of the fish which had been immersed in lower concentrations died in about 3 days after transfer to fresh water, though a few recovered. Very few of the fish which had been immersed in solutions of potassium dichromate survived on being transferred to fresh water; those that did so were usually the fish which had been the first to overturn, suggesting that these fish were affected by the acidity of the solution rather than by any specific toxic effect of the chromate.

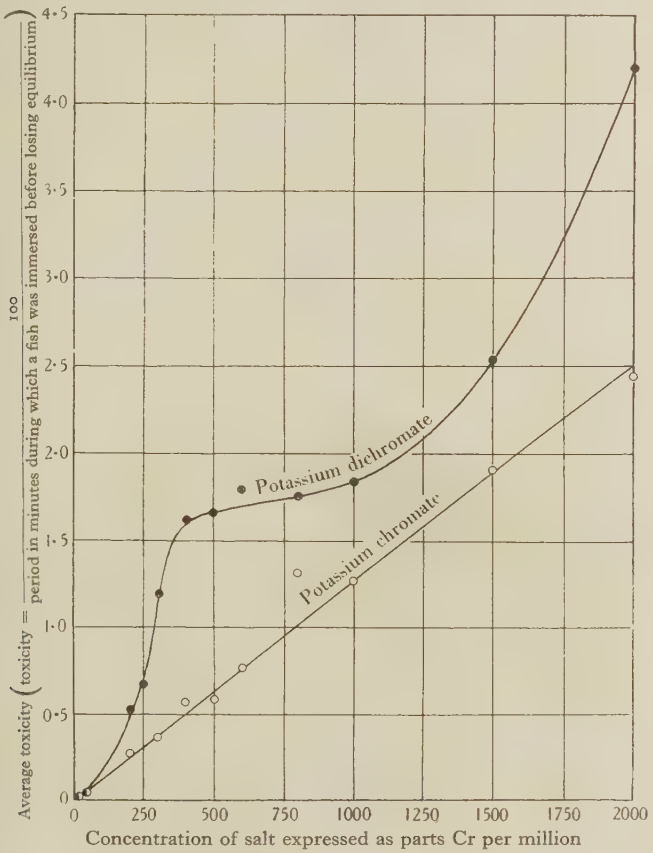

Fig. 3. Toxicity to rainbow trout (*Salmo gairdneri* Richardson var. *shasta*) of solutions of potassium chromate and potassium dichromate in distilled water

Ammonium chloride and ammonium sulphate

In tests of toxicity of solutions of ammonium chloride and ammonium sulphate, yearling rainbow

trout were immersed in solutions of the two substances in distilled water, in Watford tap water, and in mixtures of various proportions of distilled water and tap water; the water was aerated for about 16 hr. before the salt was dissolved in it. Temperature of the solutions was controlled at 18° C. during the tests. Results of the tests are given in Table 3 and are shown graphically in Fig. 4, where the toxicity of ammonium chloride and ammonium sulphate in various types of water is plotted against the concentration of salt.

When equivalent amounts of ammonium chloride and ammonium sulphate were dissolved in equal volumes of the same type of water, there was no appreciable difference between the toxicity to rainbow trout of the two solutions; solutions of the two substances were much more toxic when hard water was used than when distilled water was used. These

TABLE 3. *Toxicity to yearling rainbow trout* (Salmo gairdneri *Richardson var.* shasta) *of solutions of ammonium chloride and ammonium sulphate*

Number of fish immersed 10. Temperature of test solution 18° C.

Salt	Solvent	Test solution: Concentration (p.p.m. NH_3)	Test solution: Concentration (p.p.m. NH_4Cl)	Test solution: Initial *p*H	Toxicity*: Mean	Toxicity*: Standard deviation	Mean immersion period (min.) before overturning
Ammonium chloride	Watford tap water	1000	3140	6·9	3·66	0·42	27·3
		150	471	7·7	1·63	0·22	61·5
		100	314	7·6	1·91	0·32	52·5
		75	236	7·8	0·70†	—	143
		50	157	7·7	<0·1‡	—	>1000
Ammonium chloride	3 vol. Watford tap water *plus* 1 vol. distilled water	1000	3140	7·3	2·99	0·66	33·4
		750	2360	6·5	3·61	0·72	27·7
		500	1570	7·1	2·83	0·50	35·3
		250	785	7·4	2·86	1·02	35·0
		200	628	7·2	1·83	0·35	54·6
		150	471	7·5	1·37	0·25	73·0
		100	314	6·6	<0·023	—	>4320
Ammonium chloride	Equal vol. Watford tap water and distilled water	1000	3140	7·3	2·74	0·49	36·5
		750	2360	6·3	3·03	0·57	33·0
		500	1570	6·9	2·47	0·44	40·5
		250	785	7·2	2·37	0·46	42·2
		200	628	7·0	1·19	0·30	84·4
		150	471	7·3	0·60	0·25	167
		100	314	6·5	<0·023	—	>4320
Ammonium chloride	1 vol. Watford tap water *plus* 3 vol. distilled water	1000	3140	7·3	1·73	0·37	57·8
		750	2360	6·5	2·10	0·48	47·6
		500	1570	6·5	1·64	0·37	61·0
		250	785	6·5	1·27	0·21	78·7
		200	628	6·6	0·063	0·05	1585
Ammonium chloride	Distilled water	3000	9420	5·2	0·342	0·080	292
		2000	6280	5·7	0·404	0·113	248
		1000	3140	6·0	0·138	0·040	725
		500	1570	5·6	0·098	0·048	1020
		100	314	6·3	<0·023	—	>4320
		(p.p.m. NH_3)	(p.p.m. $(NH_4)_2SO_4$)				
Ammonium sulphate	Distilled water	3000	11640	5·4	0·314	0·064	318
		2000	7760	5·5	0·374	0·077	267
		1000	3880	5·6	0·128	0·049	847
		100	388	5·9	<0·017	—	>5760
Ammonium sulphate	Watford tap water	1000	3880	7·1	3·36	0·50	29·8

* For explanation, see text.

† Six fish overturned during immersion for periods up to 135 min.; four fish were apparently unaffected by immersion for 30 hr.

‡ One fish overturned after immersion for 217 min.; nine fish were apparently unaffected by immersion for 30 hr.

conclusions are in agreement with the findings of the other workers. Wells (1915) found that ammonium chloride in a concentration equivalent to 170 p.p.m. NH_3 killed bluegills in 4 hr. 45 min. when the salt was dissolved in hard water; in distilled water the corresponding period was 18 days. Ellis (1937) concluded that the toxicity of ammonium compounds increases by at least 200% as the *p*H value of the solution is increased from 7·4 to 8·0. The limiting concentration below which solutions in distilled $(NH_4)_2SO_4$ (68 p.p.m. NH_3) killed goldfish in 6 days or less. The threshold concentration for the immobilization of *Daphnia magna* by ammonium chloride and ammonium sulphate dissolved in Lake Erie water are stated by Anderson (1944) to be less than 134 p.p.m. NH_4Cl (43 p.p.m. NH_3) and less than 106 p.p.m. $(NH_4)_2SO_4$ (28 p.p.m. NH_3).

Trout immersed in solutions of ammonium chloride and of ammonium sulphate showed no

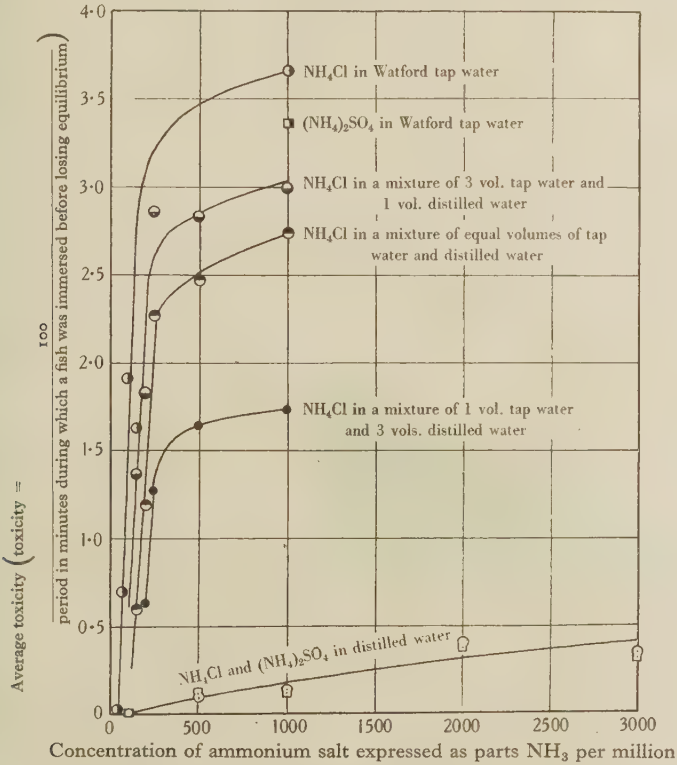

Fig. 4. Toxicity to rainbow trout (*Salmo gairdneri* Richardson var. *shasta*) of solutions of ammonium chloride and ammonium sulphate in mixtures of Watford tap water and distilled water

water of ammonium chloride and ammonium sulphate are non-toxic to rainbow trout is probably equivalent to slightly less than 100 p.p.m. NH_3; at this concentration some fish were apparently unaffected by immersion for 4 days. In tap water ammonium chloride was found to be slightly toxic to trout in a concentration equivalent to 50 p.p.m. NH_3. Clark & Adams (1913) found that 180 p.p.m. NH_4Cl in tap water (57 p.p.m. NH_3) had no effect on shiners and carp, and Ellis (1937) found that 268 p.p.m. NH_4Cl (85 p.p.m. NH_3) in hard water killed goldfish in 6 days; he also found that ammonium sulphate dissolved in hard water in a concentration of 264 p.p.m. particular symptoms of distress. The time of overturning was well defined. Only a small proportion of the fish that had overturned recovered on being transferred to fresh water.

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A method of estimating the numbers of soil Protozoa, especially amoebae, based on their differential feeding on bacteria

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(With Plate 8)

Cultural methods of estimating protozoal numbers in soil based on a dilution technique have suffered from two main sources of error: first, the use of too few replicate cultures and, secondly, the uncertain quality of the bacterial food supply. A method is described of using glass cells to set up 8 replicate cultures in one Petri dish, employing pure bacterial cultures spread over a non-nutrient agar base to provide a standard and suitable food for the Protozoa. The selection and testing of suitable bacterial strains as food supply is described. Tests for the 'recovery' of counted suspensions of Protozoa added to sterilized soil were made; the results of these are given and the limits of experimental error in the method are discussed.

1. INTRODUCTION

It has been established by various workers that large numbers of amoebae and flagellates, both active and encysted, are present in normal soil. There are two principal methods† used to estimate numbers of soil Protozoa: (1) Direct microscopic examination of a soil suspension: this has failed to give a reliable picture of the normal protozoan population. (2) A modification of the dilution method used in bacteriology. A suspension is made of the soil sample, and successively diluted. Uniform volumes of each dilution are added to nutrient media and the population calculated from the presence or absence of protozoal growth at various dilutions. Two modifications of this method have been tried.

* This work was made possible by a grant from the Agricultural Research Council, to whom the author's thanks are due.

† For an account of the previous literature the reader is referred to the papers of Cutler (1920) and Severtzoff (1924).

(*a*) *Liquid culture method*

1 c.c. portions from each of the higher dilutions of the soil sample were added to liquid media and the number of Protozoa calculated, assuming that they were well separated and that a single individual was present in the final dilution in which growth took place.

(*b*) *Agar plate method*

Cutler *et al.* (1922) placed in duplicate 1 c.c. portions of soil suspension at a suitable range of dilutions (1/25–1/204,800 or more) on to nutrient agar, and after incubating the plates for 1–3 weeks, estimated the number of Protozoa per gram of soil by counting the number of plates without Protozoa at each dilution. The statistical considerations upon which this method is based were discussed by Fisher (1922). This is the only published method that has been statistically approved.

It is limited in accuracy as compared with bacterial count methods by the difficulty of obtaining suffi-

cient replication for estimating numbers of Protozoa in soil, where a wide range of dilutions must be tested, since the numbers of Protozoa per gram of soil may vary from a few thousand to several hundred thousand (Cutler *et al.* 1922). To cover this range 15 dilutions must usually be tested, and as each replicate involves the use of a separate Petri dish the examination of the necessary sample of 15 dilutions with 2 replicates requires 30 Petri dishes. This number is doubled when a separate estimate of cystic and active forms is made. A further source of error is introduced by the difficulty of exploring the whole surface of every plate, so that reliance must be placed on samples in which it is easy to miss the presence of small numbers of Protozoa.

A further source of error in the method, more disturbing because its magnitude is unknown, is that the source of bacterial food supply is a random collection of such bacteria, derived from soil suspension, as may happen to grow on the plate.

The writer has studied, for more than 5 years, the selectivity in bacterial food by soil Protozoa and other micro-organisms. A part of this work has already been published (Singh, 1941*a*, *b*; 1942*a*, *b*). Amoebae are very selective in their choice of bacterial food; some bacterial species are readily eaten, others reluctantly, while some of them are inedible. Many soil bacteria are not only inedible to Protozoa but produce secretions actively toxic to them (Singh, 1945). Hence a protozoan in a given sample may fail to grow or may be killed if it lies on a portion of a plate amongst inedible or toxic bacteria or toxic fungi.

This difficulty can be met by using a non-nutrient agar which checks the growth of bacteria derived from the soil and by supplying a uniform edible bacterial food in the form of a pure culture (Severtzoff, 1924). The use of this culture depends on its being edible by all the Protozoa to be counted. A bacterial species readily eaten by one species of amoeba was found to be also eaten by other soil amoebae and by the common soil flagellates and ciliates (Singh, 1941*a*, *b*; 1942*a*). But to confirm this, pure cultures of 11 strains of soil bacteria were supplied as food to 9 species of soil amoebae, to the flagellate *Cercomonas crassicauda* and to the ciliate *Colpoda steinii*. The results are shown in Table 1, where the bacteria are classified according to whether they were readily eaten (RE), more slowly but completely consumed (CE), partly consumed (PE) or not eaten (N). The first 6 bacterial strains were completely consumed by all the 11 Protozoa. It thus seems reasonable to use any of these 6 bacteria* or a mixture of them in a counting method with some confidence that they would supply food to any common soil Protozoa.

* Comparative counts of soil amoebae obtained by using some of these 6 bacterial strains as food are given in Table 3.

The need for further replication as well as the disadvantage of having to rely on samples taken from the plates are met in the present method by replacing the whole Petri dish as a unit of replication, by a small disk of bacterial growth (or 'bacterial circles')* spread over the agar surface and enclosed in a glass ring. Eight of these can be fitted into each Petri dish. Moreover, the entire surface of each bacterial circle can readily and rapidly be explored for Protozoa. Thus the error involved in sampling the plate surface is obviated.

2. General description of the ring method

Eight glass rings (each 2 cm. internal diameter, 1 cm. depth and 1–2 mm. thickness) are arranged in each Petri dish (Pl. 8, figs. 1, 2). A suitable number of Petri dishes each containing 8 rings are sterilized and 1 % agar† containing 5 g./l. NaCl is poured into each of the rings as a layer about 1–2 mm. thick. If the edges of the rings in contact with the bottom of the Petri dish are not smooth, it is better to pour about 15 c.c. of agar into each Petri dish and then place 8 sterile glass rings in each Petri dish before the agar solidifies. A thick suspension of one or more readily edible species of bacteria is spread over the agar surface in each ring as a circular patch or 'bacterial circle'.‡ The bacteria are taken from slope cultures 2–7 days old, usually on peptone agar. The plates are now ready for inoculation.

The dilutions from a soil sample are made as follows: 10 g. of the sifted soil are shaken for 4 min., with 50 c.c. of normal salt solution, giving a dilution of 1/5. From this, a series of twofold dilutions are made, ranging from 1/10 to 1/81,920. In order to make each dilution 5 c.c. of next higher dilution is added to 5 c.c. of sterile normal salt solution con-

* Attempts to use 'bacterial circles' without surrounding each with a glass ring were unsuccessful, whether these were made on the agar surface or in depressions cut out of the agar and lined with a thinner agar film. In all such attempts the amoebae readily migrated from one circle to the next. This difficulty must render unreliable the method described by Severtzoff (1924).

† Agar containing only NaCl but no added nutrient is used in order to check the growth of the harmful organisms from soil inoculum. Best results are obtained if one uses agar washed for 5–7 days in distilled water, changing the water twice or three times daily, because this further reduced the growth of soil micro-organisms coming from the inoculum. 1 % agar is the best because in addition to amoebae it allows the development of flagellates in the presence of suitable bacterial food. Flagellates do not grow well on thicker agar. Lower percentages of agar, though still better for the growth of flagellates, are not very satisfactory as the gel is so soft as to make the spreading of bacteria difficult.

‡ When a large number of plates are being prepared, time can be saved by making a very thick paste-like bacterial suspension and adding a drop of this to each ring.

tained in a test-tube. Eight rings are inoculated with 0·05 c.c. of each of these dilutions.

To make soil dilutions it is necessary to use a separate pipette for each dilution, but for adding the 0·05 c.c. to the rings the same micropipette can be used if one starts from the highest dilution.

After inoculating the rings with various dilutions of soil the plates are incubated for 15 days at 20–21° C. without turning them over. After 6–7 days they are examined by turning them over under a low

method of obtaining the estimate of numbers. With twofold dilutions the standard error of the number of negative cultures is the square root of the number of replicate cultures at each level and a difference between two such estimates similarly obtained will be significant at the 5% level if the number of negative cultures differ by $1{\cdot}96\sqrt{(n_1+n_2)}$, where n_1 and n_2 are the number of replicate cultures used at each dilution level for the two counts. In the example* illustrated with 8 replicates at each level,

TABLE 1. *Edibility of various bacterial strains* by different species of soil Protozoa*

	Different species of soil amoebae										
Bacterial strains	Big	Small	*Naegleria gruberi*	4000	D.A.	H.R.	D.A. G.S.	A	M	Flagellate (*Cercomonas crassicauda*)	Ciliate (*Colpoda steinii*)
1912	RE	RE	RE	RE	CE	RE	RE	CE	CE	CE	CE
T20	RE	RE	RE	CE	CE	CE	RE	CE	RE	CE	CE
4000	RE	CE	CE	RE	CE	CE	CE	CE	CE	CE	CE
4752	RE	RE	RE	RE	CE	RE	CE	CE	RE	CE	CE
5945	RE	RE	CE	CE	CE	CE	CE	CE	CE	CE	CE
N16 (i)	CE	CE	CE	CE	CE	CE	CE	CE	RE	CE	CE
4039	PE	PE	CE	PE	PE	PE	CE	PE	CE	CE	CE
4031	NE	NE	PE	PE	PE	NE	PE	NE	PE	PE	CE
0312	NE	NE	PE	NE	NE	NE	PE	NE	NE	PE	—
6699	NE	PE	PE	PE	PE	NE	PE	NE	PE	CE	—
387	NE	NE	NE	NE	NE	NE	NE	NE	NE	CE	—

RE = readily eaten. CE = more slowly but completely eaten. NE = not eaten.

* The characters of these bacterial strains are given in the Appendix.

power of the microscope and noting the presence or absence of Protozoa in each ring. This examination, taking about 2 min. per plate, is sufficient for estimating the number of different groups of Protozoa, but when making quantitative studies of individual species a sample from each of the bacterial circles showing protozoan growth must usually be examined under a high power. A second examination should be made after 15 days; further incubation does not increase the counts obtained.

The number of Protozoa per gram of soil is then estimated from the count of negative cultures (i.e. showing no Protozoa) by applying Fisher's method of negative plates (see Fisher & Yates, 1943, Table $VIII_2$). For most material such as soil, having a variable content of Protozoa, a series of 15 dilution levels is needed. For the convenience of workers using the technique here described a table is given in the Appendix showing the estimated number of Protozoa per gram of soil derived from counts of negative 'cultures' in a series of 15 twofold dilutions varying from 1/5 to 1/81,920 with 8 replicates at each dilution level, with an inoculum of 0·05 c.c. added to each culture. If some other number of replicates, n, be used at each level, the number of negative cultures must be multiplied by $8/n$ before entering the table. An example of an actual count from a sample of Rothamsted soil is given in Table 2 to illustrate the

TABLE 2. *Showing a count of Protozoa from a sample of Rothamsted soil to illustrate the method of obtaining the estimate of numbers*

	Amoebae		Flagellates		Ciliates	
Dilutions	+	−	+	−	+	−
1/5	8	0	8	0	8	0
1/10	8	0	8	0	6	2
1/20	8	0	8	0	3	5
1/40	8	0	8	0	3	5
1/80	8	0	8	0	4	4
1/160	8	0	8	0	0	8
1/320	8	0	8	0	2	6
1/640	7	1	8	0	0	8
1/1,280	5	3	6	2	0	8
1/2,560	8	0	8	0	0	8
1/5,120	2	6	4	4	0	8
1/10,240	1	7	2	6	0	8
1/20,480	0	8	1	7	0	8
1/40,960	0	8	0	8	0	8
1/81,920	1	7	1	7	0	8
Totals	80	40	86	34	26	94

	Amoebae	Flagellates	Ciliates
Positive cultures	80	86	26
Negative cultures	40	34	94
No. per g. soil	41,400	70,500	377

* The upper and lower limits of a single estimate is $\pm 1{\cdot}96\sqrt{n}$, and in the present example with 8 replicates at each level these would be 34·5 and 45·5 negative cultures respectively. These are the fiducial limits.

the estimate of 41,400 amoebae based on 40 negative cultures would thus differ significantly from similarly obtained counts of $40 \pm 1{\cdot}96 \times 4$ or 48 and 32 negatives (since only integral numbers of cultures are observed, the upper limit is the estimate corresponding to 48 negative cultures, and the lower limit that corresponding to 32 negative cultures), giving estimates of 20,500 and 84,200. Had the estimate of 41,400 been derived from a similar dilution series with only 2 replicates at each level, it would have been based on 10 negative cultures and would have differed significantly from similarly obtained counts of $10 \pm 1{\cdot}96 \times 2$ or 14 and 6 negatives, giving estimates of 10,200 and 175,000. Thus the increase in replication greatly reduces the limits of uncertainty that

TABLE 3. *Estimates of numbers of amoebae in various soil samples from dilution plates supplied with three strains of bacteria*

Soil sample from	Counts using bacterial strains 1912	5945
Rothamsted garden	116,440	73,810
Hen run	39,820	47,440
Harpenden garden	25,950	25,950
Compost heap	25,950	21,630
Great Harpenden Field	8,833	6,139
Potato plot	6,139	5,265
Means	37,189	30,056

Soil sample from	Counts using bacterial strains 1912	4000
Garden	58,380	31,920
Allotment	21,830	31,920
West Barnfield	8,830	7,360
Fosters Field	8,830	6,140
Hoos Field	6,140	5,270
Great Knott	3,600	3,600
Little Hoos	2,945	2,945
Means	15,794	12,751

attend the estimate, and, now that higher replication has been shown to be practicable, we should regard 8 replicates as a minimum.

3. PARTICULAR POINTS IN THE TECHNIQUE

(1) *The use of different strains of bacteria as food*

Out of the 11 strains of bacteria, tests of which are given in Table 1, 6 are completely eaten by different species of soil amoebae, a soil flagellate and a soil ciliate. In pure culture work it has been found that strain 1912 (an *Aerobacter*) is one of the most readily edible species by soil amoebae, flagellates and ciliates and all the cultures of Protozoa have been maintained on it for several years. Hence this species has been used as a control against which to compare the edibility of other bacteria and their suitability for use in the counting of Protozoa.

Table 3 gives the total numbers of soil amoebae found by the present method in various soil samples by using the bacterial strains 5945 and 4000 as food supply on the plates, each estimate being compared with that obtained with strain 1912.

These three bacterial strains, when used as food supply, gave estimates in satisfactory agreement in counts from a variety of soils. This indicates the absence of any bias due to specificity in feeding habits of soil Protozoa, provided a suitable bacterial strain be used.

(2) *The separate estimation of encysted Protozoa*

To obtain estimates of encysted Protozoa in a soil sample the active forms must be destroyed without damaging the cysts. Two methods for doing this have been proposed: (1) heat and (2) HCl treatment. Cutler (1920) developed the acid treatment after finding the heat treatment to be unreliable. However, Severtzoff (1924, p. 155) says: 'Better seems to

TABLE 4. *The effect of heat and* 2% HCl *on the count of cystic amoebae*

Treatment	Cysts estimated per g. soil
Heated to 65° C. for 15 min.	70
Control treated with 2% HCl	5080
Heated to 65° C., cooled and kept at 60° C. for 15 min.	20
Control treated with 2% HCl	5540
Heated to 58° C. for 10 min.	317
Heated to 58° C. for 30 sec. and quickly cooled	1170
Control treated with 2% HCl	3600

be the method employed by us and based on Cunningham's (1913) study of the effect of heat on the active forms of Protozoa and cysts. To estimate the number of cysts and of active forms of amoebae separately, we heat the tubes with all different dilutions just used for the first quantitative analize in the water bath to 65–70° C.; in that way we kill all active Protozoa, including amoebae, and keep alive only the cysts.' The method of Cutler (1920) of using 2% HCl to kill active Protozoa has long been used at Rothamsted and the evidence that the number of cysts added to soil can be quantitatively recovered by this method is given in his paper. But in view of the above statement of Severtzoff (1924) the two methods of killing active forms were compared in the following experiment.

Fifteen twofold dilutions of soil suspensions in test-tubes were put into a water-bath and heated to various temperatures and for various lengths of time, as shown in Table 4. Test-tubes of the same thickness were chosen so that the heat in all the soil suspensions was uniform. The HCl method was used as described by Cutler (1920). After the treatments,

the numbers of cysts were estimated by the ring method, using 8 replicates at each dilution level.

Heating the soil suspensions even to 58° C. kills many of the encysted Protozoa in addition to all the active forms. The heating method is thus very unsatisfactory as a means of distinguishing encysted from active Protozoa.

That the treatment does not destroy any appreciable number of encysted forms is shown by the following experiment. Equal volumes of a suspension of the cysts of *Naegleria gruberi* formed in 7 days old culture on non-nutrient agar with *Aerobacter* (strain 1912) as food were added to each of four flasks containing 10 g. sterilized Rothamsted soil. Dilution counts were made at once from two flasks without acid treatment; the other two flasks were treated overnight with 2% HCl and dilution count plates set up the following day. The estimated numbers of cysts were as follows:

Sample	A (without acid treatment)	77,100/g.
	B ,,	101,000/g.
	C (with acid treatment)	77,100/g.
	D ,,	92,000/g.

The fact that the acid treatment does, however, destroy the active forms is shown by some of the tests described in the next section.

4. Tests of the ring method

(1) '*Recovery*' *of counted suspensions*

In order to test the ring method suspensions of the soil amoebae were counted on a haemocytometer and incorporated in known amounts in sterilized soil, from which estimates of numbers of amoebae were then made by the ring method. In the first six experiments the dominant soil amoeba *Naegleria gruberi* was used and in Exp. VII another unnamed species. The amoebae used in Exps. I and II were encysted,* those in Exps. III and IV were active, while those in the remaining three experiments included both active and encysted forms. In Exp. V separate estimates were obtained of total and of encysted amoebae, the latter after treatment of the soil with 2% HCl overnight. The results of these 'recovery' tests are summarized in Table 5 which gives the haemocytometer counts, the numbers estimated by the ring method, usually from several soil samples, with the percentage 'recovery' which these constitute. In addition, column 4 gives the numbers of negative cultures that would have given

Table 5. '*Recovery*' *of counted suspensions of amoebae added to soil*

Exp. and sample	Cyst or active	Haemocytometer count	Expected negative cultures	Actual negative cultures	Difference	Number of replicates *n*	Fiducial limits*	Estimated numbers	Percentage haemocytometer count
III *a*				34					
b				31					
Mean	A	112,560	58	65	7	16	7·76	81·250	72·2
IV *a*				31					
b				29					
Mean	A	140,280	52	60	8	16	7·76	101,000	72·0
VI	A+C	9,240	57	62	5	8	5·55	6,040	65·4
VII	A+C	7,360	60	65	5	8	5·55	4,670	63·5
V *a*				37					
b				40					
Mean	A+C	67,200	70	77	7	16	7·76	47,700	71·0
V *a*				46†					
b				48†					
Mean	C	34,080	84	94	10	16	7·76	22,500	66·0
I *a*				29					
b				31					
Mean	C	138,900	52	60	8	16	7·76	101,000	72·7
II *a*				33					
a				30					
b				33†					
b				31†					
Mean	C	132,000	108	127	19	32	11·09	86,500	65·5

* The 5% fiducial limits of a *single* estimate are $1{\cdot}96\sqrt{n}$ where n is the number of replicate plates at each dilution.
† Treated with 2% HCl.

* The cysts formed in cultures of 4 days or more on non-nutrient agar with strain 1912 as food were used. The amoebae were suspended in normal NaCl for haemocytometer counts.

estimates similar to the haemocytometer counts, column 5 the actual negative cultures observed and column 6 the difference from expectation. The fiducial limits in negative cultures for each mean estimate are given in column 8. The observed number of negative cultures was always higher and the estimated numbers therefore lower than expectation, though in Exps. III, IV, V and VII (A+C) the differences were not significant. The percentage 'recoveries' shown in the last column cover the range of 63·5–72·2% with a mean of 68·54. This rather consistent loss, which occurs with active and encysted forms suggests a systematic error such as would be produced if a percentage of the amoebae were not viable when introduced into the rings. To check this possibility individual amoebae,* both active and encysted, were planted, each in a separate bacterial circle, and the percentage that multiplied was recorded. The following results were obtained:

	Exp.	Amoebae planted	No. multiplying	% viability
Active amoebae	I	59	51	86·4
	II	66	59	89·3
	III	57	51	89·4
Encysted amoebae:				
Cysts from 8 days culture	IV	64	52	81·1
,, ,, 8 ,, ,,	VII	63	54	85·7
,, ,, 10 ,, ,,	V	48	26	54·1
,, ,, 10 ,, ,,	VIII	63	34	53·9
,, ,, 24 ,, ,,	VI	96	83	86·4
Total (A+C)		516	410	79·4

It does not seem that the cysts formed recently in the culture have a higher percentage of viability than those left for several days before being tested, but it it possible that the viability of cysts formed under different cultural conditions and with different bacterial food supply may differ. The loss of viability in culture can reasonably account for a 20% loss in 'recovery', the remaining 10% being perhaps due to injury in preparation. Thus the population of amoebae in soil is probably under-estimated in the ring method by about 30%. This error is likely to be inherent in any method of counting based on culture.

Discussion

Most of the cultural methods proposed for enumerating Protozoa have depended on the use of some nutrient medium. The fact that the various Protozoa tested all show a marked specificity in their food requirements implies that no nutrient medium that can encourage bacterial growth is reliable for use in such methods for estimating the numbers of Protozoa in a natural environment having a mixed bacterial population. Any nutrient medium may induce the growth of bacteria that are inedible or of bacteria and fungi producing products toxic to the Protozoa.

Protozoologists have indeed obtained differing estimates of Protozoa from the same sample by using media containing different nutrients such as soil extract, nutrient agar, hay infusion, etc. (François Perey, 1925; Sandon, 1928; Cutler, 1933; Dixon, 1937). These results can now be explained as being probably due to the selective encouragement of bacterial species by the different media. To avoid this, a non-nutrient substrate and a food source consisting of a pure culture of a suitable edible bacterium seems necessary if reliable estimates are to be obtained.

Choice of suitable bacterial food must be the result of feeding tests on the Protozoa to be counted. The bacterial strains selected in the present work were shown to be edible by a wide range of soil Protozoa and give corroborative results when used for estimates of protozoal numbers. Nevertheless, in a different environment Protozoa with other food requirements may be found and another selection of food bacteria would then be needed for use in estimating their numbers. In making this selection the edibility of a range of bacterial species can easily be determined by the method already described (Singh, 1941*a*). The satisfactory edible bacteria for use in estimating protozoal numbers belong to strains of small cocci or small non-sporing rods. Large rods and cocci are usually unsuitable food for the development of small flagellates. It is safer to use gram-negative organisms, because they do not encourage the growth of lysogenic actinomycetes which are abundantly present in various soils.

With the use of suitable bacterial food the method here described is applicable to a wide range of environments such as compost, sewage sludge, sea or fresh-water mud, for estimating the numbers of Protozoa that feed on bacteria. It should therefore be of use to workers in fields other than that of soil microbiology.

My best thanks are due to Dr H. G. Thornton, F.R.S., and Miss L. M. Crump for their never-failing interest in this work.

* The amoeba used in these experiments was *Naegleria gruberi*. It was grown on bacterium 1912 and the viability tests were carried out on the same bacterium.

APPENDIX

Estimates of Protozoa numbers per gram of soil from a dilution (twofold) series of 15 levels ranging from 1 in 5 to 1 in 81,920 with 8 replicates at each level and with an inoculum of 0·05 c.c. per culture (computed from Fisher & Yates, 1943, Table VIII$_2$).

No. of negative cultures	Organisms per g.	No of negative cultures	Organisms per g.	No of negative cultures	Organisms per g.	No of negative cultures	Organisms per g.	No. of negative cultures	Organisms per g.
4	1,690,000	27	132,000	50	17,300	73	2,330	96	317
5	1,430,000	28	121,000	51	15,800	74	2,140	97	290
6	1,230,000	29	110,000	52	14,500	75	1,960	98	265
7	1,060,000	30	101,000	53	13,300	76	1,800	99	243
8	931,000	31	92,000	54	12,200	77	1,650	100	223
9	824,000	32	84,200	55	11,100	78	1,510	101	203
10	729,000	33	77,100	56	10,200	79	1,390	102	185
11	650,000	34	70,500	57	9,380	80	1,270	103	169
12	581,000	35	64,500	58	8,570	81	1,170	104	154
13	520,000	36	59,000	59	7,860	82	1,070	105	140
14	467,000	37	54,000	60	7,210	83	979	106	126
15	421,000	38	49,400	61	6,600	84	898	107	113
16	380,000	39	45,200	62	6,040	85	823	108	101
17	344,000	40	41,400	63	5,540	86	755	109	90·2
18	311,000	41	37,900	64	5,080	87	693	110	79·4
19	282,000	42	34,700	65	4,670	88	635	111	69·5
20	256,000	43	31,800	66	4,280	89	582	112	60·2
21	232,000	44	29,200	67	3,920	90	534	113	51·3
22	211,000	45	26,700	68	3,600	91	490	114	42·9
23	192,000	46	24,500	69	3,300	92	450	115	34·8
24	175,000	47	22,400	70	3,020	93	412	116	27·4
25	159,000	48	20,500	71	2,770	94	377		
26	145,000	49	18,800	72	2,540	95	346		

The table* is constructed for estimating the number of Protozoa from the total number of negative cultures when 8 replicates are used at each level. Where the number of replicates is other than 8, the number of negative cultures must be multiplied by $8/n$ before entering the table. The standard error of the number of negative cultures is the square root of the number of replicate cultures at each dilution, save at the ends of the series where it is considerably increased. Two estimates will be significantly different when their numbers of negative cultures differ by more than $1{\cdot}96\sqrt{(n_1+n_2)}$, where n_1 and n_2 are the numbers of replicate cultures at each level in the two counts. Thus, where each is based on 8 replicates a difference of 8 negative cultures will be significant.

* This table has been compiled from the Fisher & Yates table by Mr D. J. Finney, to whom the author's thanks are due.

Characters of bacteria used in experiments

The characters of bacteria 1912 (*Aerobacter*), N 16 (i) (yellow Sarcina), T 20 (yellow ochre cocci), 0312 (tawny coloured short rods) and 4031 (antimony yellow medium rods) are given by Singh (1941 *a*).

The following plant pathogens: *Phytomonas tumefaciens* (Smith & Townsend) 4752, *P. hyacinthi* (Wakker) 387 and *Erwinia carotovorum* (Jones) 5945 were obtained from the National Collection of Type Cultures, Lister Institute.

4000 and 4039 are two abundant and 6699 is a rare strain of soil bacteria.† Their characters will be published later.

† These bacteria were kindly given to me by Miss L. M. Crump.

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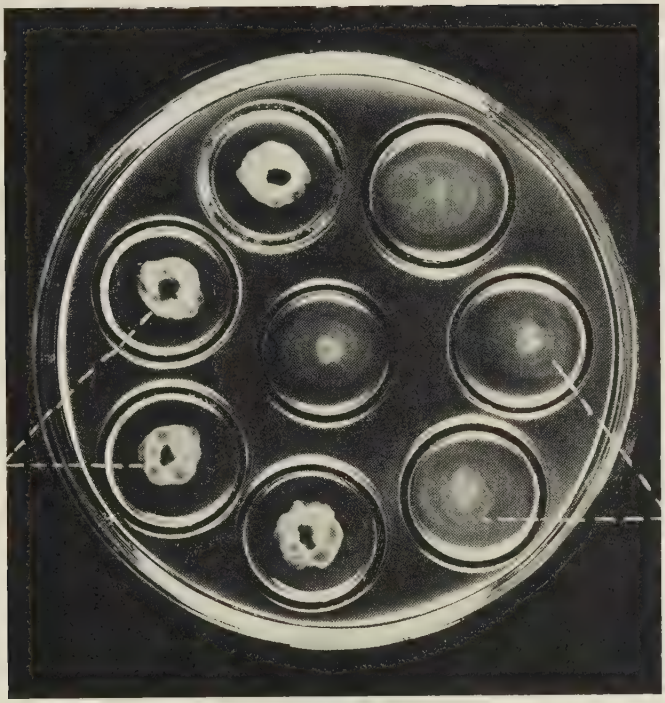
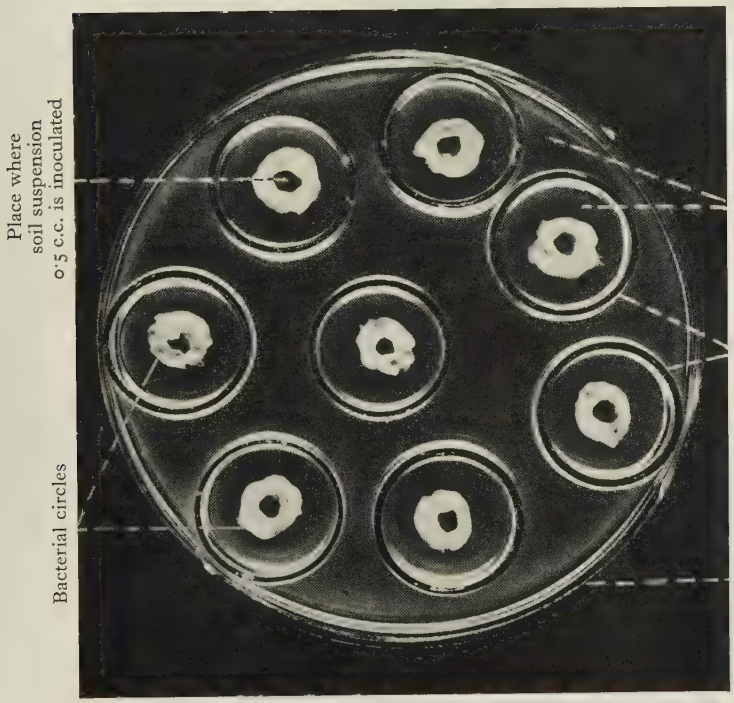


Photographs:—VICTOR STANSFIELD

Fig. 2



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EXPLANATION OF PLATE 8

Fig. 1. The plate just before inoculation of soil suspension.

Fig. 2. Four negative and four positive cultures. In the latter the bacteria (strain 1912) have been consumed by Protozoa.

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The recruitment and training of plant pathologists in Great Britain

A REPORT PREPARED BY THE PLANT PESTS AND DISEASES COMMITTEE AND ADOPTED BY THE COUNCIL OF THE ASSOCIATION OF APPLIED BIOLOGISTS

In this memorandum the term 'Plant Pathology' is interpreted in its widest sense to include *all* disorders of plants; i.e. those caused by insect and other animal pests, by fungi, bacteria and viruses, also deficiency diseases and other physiological disturbances. A 'Plant Pathologist' is therefore defined for the purpose of this report as a worker on one or more aspects of plant pathology, as above interpreted.

1. Classes of Workers considered, their Present Training, and Recommendations for the Future

For the purpose of this survey, plant pathologists have been arbitrarily classified into two groups, Specialist Advisers and Research Workers. Although County Officers are not plant pathologists, it is essential that they shall have an adequate training in the subject.

County Officers

In this group are included those whose duties are largely of a general agricultural or horticultural advisory nature. In pre-war days such duties were undertaken by the county agricultural or horticultural officers. The posts are usually held by persons who have obtained a degree or a diploma in agriculture or horticulture, usually at an agricultural or horticultural college. The courses taken are of a general nature and plant pathology, being one of many subjects in a heavy syllabus, is often dealt with only briefly.

Experience has frequently shown that some County Officers have not had the training required for dealing with even the most straightforward problems of plant disease. In consequence, matters that they should be able to deal with themselves are referred back to the Specialist Advisers on whom an unnecessary burden is placed. In other cases problems of importance that should be referred back are passed over. It is suggested, therefore, that the level of training in plant pathology of such Officers should be raised so that they would be qualified to deal at first hand with clearly recognizable plant diseases and pests. At the same time they should be able to judge which problems demand the help of the Specialist. These qualified workers would also be better able than at present to convey to the farmer the best possible advice on particular diseases and pests.

It is recommended that plant pathology be in-

cluded in any scheme of post-graduate training for agricultural and horticultural advisory officers and that, from time to time, refresher courses should be held either at a post-graduate training centre or at the Provincial Advisory Centres so that County Officers can be kept informed of the main developments in the subject.

Specialist Advisers and Research Workers

The group 'Specialist Advisers' comprises the provincial entomological and mycological advisers and their assistants. Each adviser is at present responsible for dealing with the special problems arising within his province which usually comprises two or more counties. In addition to routine advisory duties, such advisers normally examine disease and pest outbreaks of particular interest in the field and when necessary undertake research on these problems. The group 'Research Workers' includes all other workers engaged on research in the many branches of plant pathology.

An outline of the training and recruitment of Officers of these two groups will be given under the same heading, as it is considered that the type of training should be the same for both groups, at least up to a late stage in the training period. Workers in both groups have in the past entered the field in many ways, but most commonly a degree course in Botany or Zoology and/or Agriculture has been followed by two or three years of supervised research, often for a Ph.D. degree.

It is considered that such a method of preparation is too narrow for the needs of either group, a much broader training being essential in regard to the pests and diseases of plants, to general agricultural or horticultural practice, and to methods of research. The proposals put forward below are considered to be practicable and to give recruits to the specialized branches of Plant Pathology a sound and comprehensive training for their work.

(*a*) *Preliminary Training*

The best qualification is felt to be the possession of a degree of a good standard in Pure Science; undergraduate courses should, however, be related to and inspired by field practice. The principal subjects would be Botany or Zoology (or Chemistry for a potential specialist in Plant Protection). It would also generally be true that the best candidates would be those possessing an Honours Degree, but there should be no insistence on this qualification and the possessor of a General Degree in Pure Science should equally be considered. The essential points are that the candidate should have shown himself to be a good student of science, and that he should have the outlook, and as much as possible of the background, appropriate to a man whose life's work is going to be in the country and among plants. Candidates obviously suitable for the proposed post-graduate training might be recruited from time to time from graduates in Agriculture or Horticulture.

(*b*) *Post-graduate Training*

A period of 2 years at a post-graduate training centre is recommended and it is suggested that the course of training should in general follow the proposals set out below.

The objects of the course would be to give the potential plant pathologist a good conception of the whole field of plant pathology, pests and diseases alike; a substantial experience of his own particular field both as regards the general body of knowledge and the technique of research; a reasonable background of general agricultural and horticultural knowledge; and sufficient research experience to undertake research in an efficient manner. In achieving these objects, a good deal of latitude is thought to be desirable; the precise mapping-out of the course of instruction would be the responsibility of the teaching staff carrying out the scheme.

The general outline suggested for the 2-year course is as follows:

First Post-graduate Year. This would be essentially class work which would have as its object an adequate training in general plant pathology. At the beginning of this course most students would be presumed to possess some knowledge of Mycology or Entomology, but in general the amount of this knowledge could be taken as insufficient especially from the plant pathological point of view. To ensure that *all* plant pathological workers have an adequate picture of the field of study, it is suggested that at least one term, and possibly one half-session of the first post-graduate year, should be devoted to covering the general ground. Broadly speaking, half of the time would be devoted to the botanical and half to the zoological aspects, and all students would attend both courses. It is most essential that the botanical and zoological studies should not be separated too soon, and that the entomologist or mycologist should have an adequate appreciation of each other's subject.

These courses would presumably run from October to December or March and consist largely of lectures and laboratory work. The aim would be to give a knowledge of systematics, and practice in identifying insects and fungi and in entomological and mycological techniques. There would also be lectures with practical work on insecticides and fungicides.

The second part of the year (one-half or two-thirds of the full year) would bring a partial separation of the botanical and zoological sides. The mycologists would study the incidence of diseases in crops, collect and identify plant pathogenic fungi and virus infected plants, lay out experimental plots, etc.; and similarly the entomologists would make appropriate studies. There would still be some work common to all students, for example, in the handling and application of insecticides and fungicides.

During this period a course of lectures and field practice should be arranged on certain aspects of general agriculture so as to provide adequate points of contact with agriculturists.

In the summer of this first year of training it should also be practicable to give the student his first introduction to research or advisory work. This might take the form of a preliminary study of a research problem, or might consist in the student giving assistance in one or more research or advisory projects. This aspect of the work should not, however, be allowed to encroach at this stage on the more general work outlined above.

Second Post-graduate Year. In this year some considerable time would be spent on individual research and the study of research technique, but in addition there should also be general instruction on various aspects of plant husbandry, statistics, etc. As the year progressed some segregation might arise. One student might show an aptitude for research and tend to develop in that direction; another might show a less particularized interest and become more interested in advisory work. The training of these two types of student might then suitably be directed along somewhat different lines. Nevertheless, the divergence would be so small and so late in appearing that, at the end of the 2-year period, any student could be considered a potential research worker or advisory officer. Which line he took up later would depend partly on circumstances and partly on his preference.

At the end of this period of training neither type of worker would have been able to carry on research to the standard required for a Ph.D. degree. The attainment of Ph.D. standard at this stage (i.e. 2 years after graduation) is not feasible if the broader training described above is to be given and it is strongly urged that this training is a paramount necessity. After completing his course of training, however, the student should be able, if he wishes, to proceed to a Ph.D. degree. He could either be posted as a probationer to a Research Station with facilities for working for a higher degree or to an Advisory Centre where he would work under the supervision of an Advisory Officer. Under the supervision of an Advisory Officer, there should be good opportunity for the probationer to obtain a higher degree in much the same way as the worker in a Research Station.

It might happen in exceptional cases that at the end of the 2-year period of training (which has so far involved only 1 year's research) the special work being carried on by a student (either a specific research problem or possibly survey work of a more general character) might have reached such a stage that its discontinuance would involve a sacrifice of important results or discouragement to the student. In such a case it should be possible for the work to be continued at his training centre. This would not involve any departure from the main plan, as a student so treated would merely be considered as beginning his probationary period at the Training Centre instead of elsewhere.

The requirements of a Plant Pathology Training Centre will be dealt with more fully later, but there is one point which may suitably be stressed here. The scheme requires that at training centres research on plant pathology is being actively developed in order that the student may be brought into contact with the practical type of problem. A training centre is not likely to carry out its functions adequately unless it is suitably staffed and equipped to undertake research work on important agricultural and horticultural crops.

It is suggested that the 2-year course should lead to a special diploma awarded by a suitable examining body.

2. Training Facilities

At Present

As a result of a detailed survey of the present position it is clear that the courses at present available at many Universities and Agricultural and Horticultural Colleges provide most of the preliminary training necessary for a plant pathologist. Certain of the Pure Science courses, however, give little training in field studies, and many of the Agricultural and Horticultural courses devote very little time to certain aspects of Pure Science.

Two or three Universities at present offer pre-graduate instruction in plant pathology and agricultural zoology (as distinct from pure mycology and entomology) in their degree courses, but post-graduate facilities for training (as distinct from research) are almost non-existent.

In most University Agricultural Departments and in some Agricultural and Horticultural Colleges, instruction in plant pathology to a lower standard is given in the normal degree or diploma course, but in others this is not the case. The standard required varies very considerably and in the two National Diplomas commonly taken (N.D.A. and N.D.H.) the proportion of plant pathological knowledge required is very small.

Facilities on a much more adequate scale are available at a number of centres for post-graduate research on plant pathology, but, as previously described, the training in research at such centres is often confined to the narrow limits of the problem in hand. No information has been received of any comprehensive *post-graduate training course* in the subject.

This situation compares unfavourably with the position in the United States where the facilities for the teaching of plant pathology in some Universities are on a very extensive scale. The accommodation and equipment for the study of the subject are considerably better than those of any British University. While, as described above, it is considered that the best grounding in plant pathology can be given by a

degree in Pure Science followed by special post-graduate training in plant pathology it is felt that much could be learned from a consideration of the facilities at present available in the United States.

Future Requirements

The recommendations set out above require the existence of a centre or centres adequately staffed and equipped to furnish the necessary training in plant pathology.

Each centre should form an integral part of a University in order that students shall have the benefits accruing therefrom. The centres should be located in country areas in which diverse types of crops are grown, so that a variety of economic problems can be studied at first hand. Contacts and discussions with farmers would form a valuable part of the training. Accessibility to London for attendance at meetings of scientific societies is thought to be desirable.

In addition to the facilities for class work on plant pathology there must be well-equipped entomological and mycological laboratories in which the student will receive a thorough training in all aspects of laboratory technique. It is also essential that extensive facilities shall be provided for training and research *in the field*. It must be possible for both greenhouse and field experiments to be carried out and demonstration plots maintained. Thus, the student will receive instruction in the observation of disease in growing plants, in the practical applications of methods of plant protection, and in the technique of field experimentation. Such facilities will also ensure that in any research problems undertaken by the student adequate attention can be paid to practical aspects. Close liaison should also be maintained with other research centres engaged in work on special diseases and crops, and every opportunity taken of observing diseases and pests occurring on plants grown under commercial conditions. As previously stressed, in addition to the teaching of plant pathology there must be a really active research section at the centre where research on plant pests and diseases is being carried out by senior workers.

Facilities for the study of plant husbandry should be available either within the University or by arrangement with an Agricultural or Horticultural Centre.

The combination of all the above desirable features in one institution may not be easy to attain in practice, but it is strongly felt that considerable advances in the direction indicated are possible and most desirable. To obtain the facilities suggested above it may be necessary to establish a new Plant Pathology Training Centre or Centres. The number of centres required would depend on the estimated number of plant pathologists of all types to be trained annually, and the number of these would in all probability not be large.

It has to be recognized that the establishment of a new Institute combining training with a comprehensive programme of research might be difficult in view of the existence of several Research Institutes which cover the main crops of the country. The alternative procedure would be the strengthening, in very substantial degree, of certain existing University Departments of Botany and Zoology to allow of post-graduate training in plant pathology to the extent and in the manner which this report points out to be essential.

3. Recruitment

Recruitment for the plant pathological service has, in the past, been such that workers have entered it either almost completely untrained or in a very haphazard fashion. Although too high a degree of standardization is to be deprecated, it is felt that the establishment of recognized training courses as described would facilitate recruitment.

Adequate provision must be made for scholarships to cover training and maintenance. For all types of worker, however, it is recommended that the number of scholarships given shall be related to the estimated demand for such workers, and that each scholarship should carry with it some likelihood that a satisfactory student would be assured of a position at the completion of his training. Such a scheme is already adopted by the Colonial Office in recruitment for the over-seas agricultural service. This procedure would do away with the unsatisfactory situation prevailing in pre-war years when the number of scholarships given was unrelated to the demand for workers. In consequence many promising students were prompted by a feeling of insecurity to accept any position offered, often before the completion of their training.

4. Technical Assistants in Plant Pathology

Another worker in plant pathology of whom mention should be made in this report is the *Technical Assistant*. Such workers usually perform duties of a more or less routine nature as assistants to research workers and others. No suggestions have been made for their training because they may be drawn from diverse sources and are then trained in some particular branch by their superiors. Such assistants give valuable services in plant pathological research and the provision of an adequate number of them with satisfactory conditions of employment is considered essential to the smooth and economic functioning of such work.

Occasionally, such technical assistants may, by reason of particular personal qualifications, eventually engage in research or other work on their own, and thus by-pass the preliminary training courses.

While such a method of entry into the services is felt to be an inadequate substitute for the methods already described, it is felt that no really suitable worker should be debarred from advancement in this way. Full facilities should be available for workers of the type described to strengthen their fundamental training by attendance at appropriate courses.

5. Summary of Recommendations

(*a*) One or more Plant Pathology Training Centres should be established where training and research will be directed by plant pathologists working with satisfactory laboratory and field facilities.

(*b*) The level of training in plant pathology of County Officers should be raised and refresher courses should be provided from time to time.

(*c*) Specialist Advisers and Research Workers should take a degree in pure science and then study for two years at a Training Centre. Here they should receive instruction in plant pathology, plant husbandry and related subjects and obtain experience in research.

(*d*) The training and maintenance of students should be covered by adequate financial provisions.

6. Acknowledgement

In the course of the survey of present training facilities, the Universities and other centres contributing to the training of workers were invited to give details of the courses of study normally available to students. The information supplied greatly helped in the assessment of the present position.

Proceedings of the Association of Applied Biologists

Ordinary Meeting of the Association held on Friday, 5 October 1945, in the London School of Hygiene and Tropical Medicine; the President, Dr C. B. Williams, in the Chair.

Symposium on some agricultural uses of D.D.T.

The following papers were read and discussed:

1. Introduction. By Mr C. T. Gimingham.
2. Apple blossom weevil and its control by D.D.T. By Dr G. H. L. Dicker.
3. Experiments with D.D.T. 'smokes'. By Dr M. Cohen.
4. The control of flies in farm buildings by D.D.T. By Messrs W. Steer and K. J. Coghill.
5. The control of sheep blowfly by D.D.T. dips. By Mr J. B. Cragg.
6. Experiments on the control of sheep ticks by D.D.T. By Messrs G. B. S. Heath and J. G. Mitchell.

Apple blossom weevil and its control by D.D.T.

By G. H. L. Dicker, *Research Station, East Malling, Kent*

Measures in use against apple blossom weevil before the discovery of D.D.T. gave only a partial control.

The weevil overwinters as an adult mainly under loose bark or in hedgerows. Emergence begins in late February or early March and continues for about 5 weeks. The weevil feeds by boring into the fruit buds but this causes only minor injury. Egg laying does not begin until the bud-burst stage, then continues for approximately 3 weeks. The eggs are deposited singly in young blossom buds within which the entire larval and pupal life is passed. Adults are still coming out of hibernation when the first eggs are laid and continue to do so for at least another week. Control measures must therefore be delayed until as many weevils as possible are on the trees but before egg laying begins. It is fortunate that the beginning of egg-laying coincides with the bud-burst stage, thus permitting advice to be given without reference to actual dates.

In laboratory trials a D.D.T. dust on a gypsum-china clay base proved very efficient, the kill increasing with concentration until at the 5% level an average of 90% died in all tests carried out, whether the weevils or food materials were treated. Another insecticide, benzene hexachloride, gave promising results with a kill of about 60% when used at 5% on the same base. These materials differ markedly in their rate of action; benzene hexachloride acts relatively rapidly, the total number of weevils dead and paralysed remaining constant after about 16 hr., whereas with D.D.T. the mortality curve rises steadily during the 2 days following treatment then flattens out until no further kill is recorded, usually on the fifth day.

Only dusts have been used in field trials. To prevent drift interfering with results the layouts have been kept very simple. In 1944, by dusting 76 trees in the centre of a long, narrow block on 8, 13 and 20 April with 5% D.D.T. the capped blossoms were reduced to 0·02%; controls at each end gave 4·1 and 6·6% capped blossoms. The dust was applied at 60–70 lb. per acre each time. A plot of 1½ acres, dusted for comparison with 1% rotenone, gave an apparent reduction in the capped blossoms of 50%.

Further trials in 1945 confirmed the efficiency of a D.D.T. dust. With each treatment duplicated and three controls, 0·7, 6·1 and 16·8% capped blossoms were recorded respectively for 5% D.D.T., 3% D.D.T. and controls. Two applications of each dust were made at 40–45 lb. per acre on 17 and 23 March.

Another trial which included 3% dusts of D.D.T. and benzene hexachloride gave less conclusive results. The benzene hexachloride appeared to reduce the capped blossoms by 50% whilst D.D.T. had no noticeable effect. Details of application were the same as for the other trial.

These experiments indicate that an adequate control of apple blossom weevil can be obtained with a 5% D.D.T. dust if applied at the bud-burst stage and a week later.

Measures to control the weevil take place so early in the season that it is unlikely that beneficial insects will be affected. Dusting is completed a month before the blossoms open, when only an occasional ladybird is present on the trees. Nor does D.D.T. appear to persist in toxic form for long; preliminary observations after placing sheets beneath the trees

indicate there is little mortality 11 or more days after treatment, though apparently healthy insects can be tapped from the trees.

Laboratory trials have also shown that a high rate of kill is obtained by spraying weevils with 0·05 % D.D.T. suspension in water. Similar mortality occurs if shoots are dipped in suspensions of this strength and after being allowed to drain and dry, placed in cages containing healthy weevils. In one instance the dipped shoots appeared to retain toxicity for 3 weeks.

Further trials are necessary to discover if one application of a 5 % D.D.T. dust will give adequate control; how efficient 5 % benzene hexachloride is in the field; whether D.D.T. to control the weevil can be combined with the routine petroleum spray, and the strength and number of applications necessary to effect control with straight D.D.T. sprays.

Experiments with D.D.T. smokes

By M. Cohen, *Entomology Laboratory, University of Manchester*

Fleck (1944) has shown that D.D.T. is volatile; 63·36 mg. of D.D.T. at 45° C. lost 0·34 mg. per day by evaporation for the first 4 days and 0·05 mg. per day at the end of 37 days. This slowness of volatilization may account for lack of trials of D.D.T. as a fumigant. Experiments described below show that paper suitably impregnated with D.D.T. will burn to produce a smoke which is insecticidal both before and after settling.

Method of smoke production. Squares of blotting paper and filter paper were first soaked in D.D.T. dissolved in acetone or benzene, then dried, again soaked in 5 % KNO_3 to ensure even smouldering and re-dried at 50° C. The prepared paper was held in a closed cylinder 18 × 4 cm. and ignited by contact with an electrically heated resistance wire. A heavy smoke resulted which almost all settled in 20 min. Samples of smoke, taken up in benzene which was then evaporated, gave a crystalline residue agreeing in melting-point with D.D.T.

Effect on insects. Houseflies (*M. domestica* L. and *F. canicularis* L.), the stable fly (*S. calcitrans* L.), drosophilid flies, mosquitoes (*Culex* spp.), thrips, clothes moths, pollen beetles (*Meligethes aeneus* F.) and various aphids were subjected in cylinders to the fumes of smouldering D.D.T.-impregnated paper. All were knocked down in 5 sec., excepting the aphids, which remained on the foliage inserted into the chamber before the ignition. They withdrew their mouthparts from the leaves and were readily dislodged by jarring. Leg movement returned after 10 min. and the aphids attempted to walk. Numbers of *Capitophorus fragariae* Theob. were still moving 15 hr. after exposure to the smoke, but none of 25 aphids on a 3 × 1 in. slide had been able to walk off. Houseflies remained motionless until the fumes had almost all settled. Then unco-ordinated leg movements occurred and death followed after several hours.

In thrips, leg movement also returned after 10 min. but they were not able to walk off a surface and died in 8–9 hr.

Smokes produced by burning paper soaked only in KNO_3 did not produce any insecticidal effect.

Deposits from burning D.D.T.-impregnated paper. It was observed that a deposit of minute droplets formed on glass held over the smouldering D.D.T.-impregnated paper. If such deposits were stroked lightly with a camel-hair brush or by the feet of insects they behaved like a supersaturated solution, the tracks being marked by the formation of crystals.

Surfaces carrying the deposit had the same effect on insects as those sprayed with preparations of D.D.T. Thus in one trial a cylinder was fumigated with 1·6 sq.cm. of paper which had been soaked in a 5 % solution of D.D.T. (82 %) in acetone. The paper burnt contained about 0·13 mg. of D.D.T. The volume of the cylinder used was 226 c.c. and its internal area 276 sq.cm. After the fumes had settled, two *F. canicularis* and one *S. calcitrans* were introduced and all were knocked down in 20 min. After 16 days the deposit in this cylinder remained insecticidal, knocking down *F. canicularis* in 22 min.

Another cylinder was fumigated with 1 sq.cm. of a paper impregnated with 2·7 mg. D.D.T. in benzene. When the fumes had settled, one *F. canicularis* and two *M. domestica* were introduced. The former species was knocked down in 9 min. and the latter in 17–19 min. Flies were still killed by this residue 16 days after deposition. On the hypothesis that no D.D.T. is lost in combustion, 0·01 mg. was present per sq.cm. of the cylinder wall.

Large-scale fumigation. An outbuilding $5\frac{1}{2} \times 4 \times 6$ ft. with a wall area of 150 sq.ft. was fumigated with 9 sq.ft. of paper containing 2·5 g. D.D.T. per sq.ft. The paper was in 20 sheets set upright on the floor 1 ft. apart. Sheets of glass were arranged on the walls, floor and ceiling and on a bench. The papers were ignited and after the fumes had settled the sheets were removed for analysis. The heaviest deposits were found on horizontal surfaces or near the floor, the average being 17·7 mg./sq.ft.

A preliminary experiment suggested that the direct heating of D.D.T. in a greenhouse gave volatile products which were insecticidal but not phytotoxic.

It would appear that there is a field for further work on the production of smokes and vapours of D.D.T. It may not be possible to obtain heavy deposits and the processes may involve much wastage of the material. Nevertheless, if the fumes or vapour alone prove insecticidal, the development of the method for fumigation may prove valuable even though the lasting toxicity of the deposit is not utilized. Such applications can be envisaged in treating glasshouses, granaries, stores and ships' holds.

The writer is indebted to the Geigy Co., Ltd. for the suggestion of burning impregnated paper and for help in the analysis of the deposits.

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The control of flies in farm buildings by D.D.T.

BY W. STEER, *Advisory Entomologist, Manchester University* and K. J. COGHILL

TABLE 1. *Extract from counts of fly population:* 1944 *trial*

		Skylight		4th stall		Per bucket		Settled per stall		Flying per stall		Per sq.yd. of wall		Dead in feeding passage	
Date	Time	W	E	W	E	W	E	W	E	W	E	W	E	W	E
31 July	3.00 p.m.	50	25	45	40	10	4	35	12	18	35	2	4	—	—
2 Aug.	9.30 a.m.	25	25	25	15	5	8	20	24	15	18	2	6	—	—
	3.00 p.m.	50	40	110	55	10	10	70	35	35	35	4	6	—	—
	3.10 p.m.	75	50	35	25	5	5	33	25	10	10	1	1	—	—
	4.00 p.m.	100	90	22	20	3	8	25	25	5	5	0	2	—	—
	7.30 p.m.	40	40	25	13	5	2	20	12	10	10	1	1	—	—
3 Aug.	9.30 a.m.	30	20	8	8	5	3	5	8	5	8	2	2	—	—
	3.00 p.m.	40	20	70	85	12	12	35	45	25	25	2	1	—	—
	3.10 p.m.	65	55	25	70	5	10	15	25	15	12	1	1	—	—
	7.30 p.m.	60	55	20	15	8	4	12	8	8	10	2	3	—	—
4 Aug.	*Western shippon sprayed* (10.30–12.15 a.m.)														
	3.00 p.m.	12	25	4 (50)	35	2	12	5	22	5	12	0	2	250	—
	3.10 p.m.	50	55	3	22	2	6	3	22	3	12	0	2	—	—
	4.00 p.m.	100	120	3	45	2	12	8	35	4	10	0	2	—	—
	5.15 p.m.	80	140	4 (90)	18	2	7	5	18	5	5	0	0	—	—
	5.30 p.m.	100	65	3	18	1	3	3	18	3	12	0	2	—	—
	7.30 p.m.	60	325	4	18	1	8	4	20	2	12	0	2	—	—
5 Aug.	9.30 a.m.	5	180	25	70	2	10	15	40	8	20	1	2	150	75
	3.00 p.m.	15	80	3	60	2	5	5	28	5	35	0	2	53	—
	3.10 p.m.	50	120	8	21	1	5	8	12	7	10	0	0	—	—
	4.00 p.m.	130	190	0	15	1	2	3	12	3	25	0	2	—	—
	7.30 p.m.	20	250	7	25	1	5	8	22	5	15	0	2	50	—
6 Aug.	9.30 a.m.	1	115	14 (25)	35	1	12	12	30	5	10	1	0	145	12
	3.00 p.m.	7	25	8 (10)	40	1	10	8	30	3	15	0	1	25	0
	3.10 p.m.	25	30	10	50	2	5	8	30	5	10	0	0	—	—
	4.00 p.m.	40	65	3	25	1	5	3	12	5	5	0	2	—	—
	7.30 p.m.	45	65	0 (49)	10	0	5	2	12	12	3	0	1	25	0
7 Aug.	9.30 a.m.	4	30	14 (15)	75	1	5	12	35	2	33	0	2	36	8
	3.00 p.m.	2	4	10	45	0	8	5	35	2	22	0	0	0	3
	3.10 p.m.	50	7	5	20	0	8	5	20	8	5	0	0	—	—
	4.00 p.m.	85	25	3	10	0	10	8	10	10	5	0	0	—	—
	7.30 p.m.	90	50	13 (26)	10	1	2	8	12	5	2	0	0	—	—
8 Aug.	9.30 a.m.	11	55	7 (23)	35	1	10	8	25	3	8	2	1	135	6
	3.00 p.m.	18	15	21 (10)	45	0	8	10	33	5	3	0	1	—	—
	3.10 p.m.	25	18	3	40	0	5	4	33	2	30	0	2	—	—
	4.00 p.m.	25	65	3	12	0	5	3	12	0	5	0	2	—	—
	7.30 p.m.	58	98	4	6	1	6	9	9	4	7	0	0	—	—
9 Aug.	9.30 a.m.	11	106	6 (8)	15	1	8	10	25	2	15	0	1	56	10

Figures in brackets=dead and moribund flies picked up in stall

Preliminary trials by Burgess in 1943 indicated that the stable fly (*Stomoxys calcitrans*) could be controlled in farm buildings by spraying inside walls with 1 % Guesarol (Guesarol = 5 % D.D.T.).

1944 *trials.* In 1944 detailed tests were made in two adjoining identical shippons at the Cheshire School of Agriculture. Each shippon was a brick building 60 × 33 × 9 ft., in which 25 cows were milked daily. Records of fly population (see Table 1) were taken up to five times daily on a selected stall, on a skylight, per bucket and per sq.yd. of wall. The number of dead flies swept up in the feeding passages was also recorded. Preliminary counts (31 July–3 Aug.) established that infestations in the two shippons were approximately at the same level. The flies present were mainly *S. calcitrans* with *Fannia canicularis* as the second most abundant species.

On 4 August the whole interior of the western shippon was thoroughly sprayed with 2 % Guesarol using approximately 1 gal./30 sq.yd. The records following the spraying (see Table 1) demonstrated that the fly population remained lower in the sprayed than in the unsprayed shippon. Wide daily fluctuations in the records reflect weather conditions: fly activity was favoured by warmth and sunshine; depressed by cold and dullness.

1945 *trials.* In 1945 the treatments of the two shippons were reversed. Records of this trial showed that deaths in the shippon unsprayed in 1945 were three times the corresponding figure of 1944, suggesting that a residual toxicity persisted from the spraying 14 months earlier.

Throughout the two years' trials the insect population was constantly reinforced by flies brought in on the cows. Records were made from which it is estimated that a mean of 200 flies per cow was brought in daily. Very few were taken out except of the species *Lyperosia irritans*, which never leaves the cow to settle on the walls and so was unaffected by the D.D.T.

Owing to this reinforcement of population the D.D.T. treatment of the walls did not entirely prevent worrying of the cows, but it did lessen it considerably because of the marked reduction in the resident fly population of the shippon.

A subsidiary trial suggested that D.D.T. applied mixed with whitewash was equally successful in making the walls toxic to flies.

The control of sheep blowflies by D.D.T. dips

By J. B. Cragg, *Agricultural Research Council, Unit of Insect Physiology at the University College of North Wales, Bangor*

A number of Calliphorine flies, principally *Lucilia sericata* (Mg.), cause sheep myiasis in Great Britain. The fly lays its eggs in the fleece, most commonly in the tail region and given a high relative humidity, the eggs hatch and the larvae establish themselves on the flesh of the host. Under suitable weather conditions the attack proceeds rapidly because the larval excreta on the living sheep attracts more blowflies. Unless an infested sheep receives treatment, myiasis may cause death within 2–3 days.

Dipping sheep in arsenic-sulphur preparations is the principal control measure used in this country.

Promising results were obtained in 1944 using a dip (M21) containing 0·5 % D.D.T. (Cragg, 1945) and further tests were made using a similar dip (M42) in 1945. As far as possible, the experimental (D.D.T. dip) and control (proprietary dip) flocks were equal in numbers, breed and sex. Where it was impossible to divide the lambs into two equal groups, the larger portion (except in Exp. 3*a*) was placed in the experimental group. In all experiments the immersion time was 30 sec. and the control group was dipped first. Over 90 % of all sheep used were either Welsh Mountain, or Improved Welsh.

Field Trials using 0·5 % *D.D.T.* The results of the 1945 experiments are summarized in Tables 1 and 2. It is clear that under North Wales conditions, and with Welsh sheep, a D.D.T. dip surpassed the proprietary dips in controlling myiasis. Sheep dipped in 0·5 % D.D.T. remained free from strike for at least 3 weeks.

Table 1. *Field trials on the efficiency of D.D.T. as an anti-blowfly dip*

Date (1945)	Exp.	Sheep dipped		Strikes recorded	
		Control group	D.D.T. group	Control group	D.D.T. group
26 June	1	141	141	12	5
28 June	2	114	117	0	0
3 July	3*a*	352	341	9	4
4 July	3*b*	225	233	24	5
28 Aug.	4	143	149	7	0
4 Sept.	5	80	80	3	0
Totals		1055	1061	55	14

All the experiments lasted 6 weeks except no. 5, where the records cover only 4 weeks.

Mode of action of D.D.T. dips. Cragg (1945) showed that the fleece of sheep which had been

dipped in 0·5% D.D.T. remained toxic to blowflies for at least 5 weeks, and that over a period of 6 weeks complete egg batches were not laid in response to chemical attractants placed in the fleece.

It was shown that the absence of oviposition was not due to any repellent odour possessed by the dip.

Egg batches placed in wool which had been dipped in 0·5% D.D.T. hatched and some of the larvae were able to establish themselves on meat. At 34° C., 24 hr. contact with such wool was without an immediate lethal effect on the majority of 3rd instar larvae. Although such larvae could pupate, no emergence occured.* Whilst prolonged exposure to D.D.T. may be highly toxic, once larvae are feeding on the flesh of the sheep they are on a surface which should be free from D.D.T. The occurence of dead larvae in strikes found on D.D.T.-sheep may have resulted from larvicidal action, but a reduction in the amount of oviposition through

TABLE 2. *Number of days between the date of dipping and the occurrence of the first strike*

Exp.	Control group	D.D.T. group	
1	17	28	7 out of 12
2	No strikes	No strikes	—
3*a*	9	24	2 out of 9
3*b*	6	23	5 out of 24
4	3	No strikes	—
5	9	No strikes	—

The last column shows the number of strikes which had occurred on the control group by the time that the first strike was recorded in the D.D.T. group.

the action of the dip on the flies could produce the same effect. Since small strikes tend to dry out, some larvae would be killed by desiccation. Observations on the carcass of a sheep which had been dipped with D.D.T. 6 weeks before death showed that oviposition might be considerably reduced. The carcass, although highly attractive to blowflies, was very slowly colonized by larvae. Many dead flies surrounded it and the number of egg batches found in the 5 days following death was only a fraction of the number which, under similar weather conditions, would have been expected on a carcass free from D.D.T.

Effectiveness of different D.D.T. concentrations. The efficiency of D.D.T. over the concentration range 0·1–0·75% (Emulsion M42) was tested by using attractants and counting the number of egg batches laid in the region of the pad. The sheep were Herdwick and Herdwick-Swaledale crosses and the experiments were made at Cragg Farm, Cumberland. The results are summarized in Table 3. At both 0·1 and 0·25% some egg batches were laid. Whilst the amount of oviposition was less than that on the controls dipped with a proprietary arsenic dip,

* A detailed account of the action of D.D.T. on blowfly larvae will be published elsewhere.

the figures indicate that with the present type of emulsion 0·5% D.D.T. probably represents the minimal concentration suitable for use on a field scale.

DISCUSSION

Although D.D.T. gave better protection against sheep myiasis than did the proprietary dips with which it was compared, the protection was not complete, some strikes occurring on D.D.T.-dipped sheep. In recommending the material for use against the blowfly, other control measures should not be neglected. In particular, the practice of crutching should be advised (Hobson, 1941; Angus *et al.* 1944). This considerably reduces the incidence of tail strikes, and in the present experiments, which were

TABLE 3. *The efficiency of D.D.T. over the concentration range* 0·1–0·75%

Concentration of D.D.T. (%)	Days after dipping			
	3	7	14	18
0·1	0	0	—	1
	½	0	0	0
	0	0	2	0+
0·25	0	0	0	1
	0	0	1+	0
	0	0	1	0
0·5	0	0	0	0
	0	0	0	0
	0	0	0	0
0·75	0	0	0	0
	0	0	0	0
	0	0	0	0
Controls	0	1	1	1
	0	3	1	2
	0	0	4	0

The figures recorded against each concentration represent the number of egg batches laid on each of three sheep used for the test. +indicates that the eggs were scattered instead of being in a compact mass.

made on uncrutched sheep, 64 out of the 69 strikes recorded were on the tail region.

Although D.D.T. has little immediate larvicidal action, it should serve a useful purpose in maggot dressings, as a substance which will reduce the incidence of restrikes.

In using D.D.T. against the blowfly a revolution has taken place in the method of control. With existing proprietary dips, very good penetration of the fleece is essential since their effectiveness is dependent on a high deposition of larvicide on the skin. With D.D.T. the aim should be to provide a good surface covering which will be toxic against the blowfly, a deposit on the skin being of secondary importance. Insecticides of the D.D.T. type may therefore be equally well applied to sheep by means of sprays or jets rather than by dipping. Pioneer

work in the use of these alternative and certainly more hygienic methods of applying insecticides to sheep has been done by Moore (1937) in this country, and the work should be extended.

The writer wishes to thank Mr L. Davies for assistance with the field experiments and Dr J. G. Mitchell of the Chemical Research Laboratory, D.S.I.R., Teddington, who supplied the D.D.T. preparations.

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Reviews

The Potato in Health and Disease. By T. Whitehead, T. P. McIntosh and W. M. Findlay. Pp. xv + 400. Edinburgh and London: Oliver and Boyd. 1945. 25*s*. 0*d*.

This book is described as a 'second edition—revised and enlarged', and it is apparently to replace McIntosh's *The Potato, its History, Varieties, Culture and Diseases*, published in 1927 and long out of print. The book has, however, not only been completely rewritten but altered in character, and it is, to all intents and purposes, a new work.

Chapter I gives a somewhat meagre account of the economics of the potato crop. Chapter II contains a brief summary of the views of Russian and English workers on the origin, introduction and systematic position of the potato, notes on other tuberous *Solanum* spp., and a general description of the potato plant. Chapters III–V give an admirable account of intervarietal differences in the foliage, floral parts and underground structures of the plant and are followed by a useful chapter on variations and correlations. There are then five excellent but rather selective chapters on the husbandry of the crop, written from a distinctly Scottish point of view. The remaining nine chapters are devoted to the diseases of the plant: mycological detail is minimized, and although the accounts are written in a technical way the material is treated from an essentially practical standpoint. As one would expect of Dr Whitehead, the discussion of potato diseases, especially the virus diseases, is excellent, but here and there one finds statements that require a little emendation and some of the names of the pathogens and their authors need checking by the *B.M.S. List*. This disease section terminates with a useful bibliography, set out rather awkwardly by chapters; in the earlier chapters a few references are inserted as footnotes. Appendix I is a short practical account of fungicidal treatments for the control of potato diseases. Appendix II, which runs to 36 pages, contains admirably diagnostic notes on 53 common commercial varieties, a table of the reactions of 64 varieties to viruses, and descriptions of the sprouts of 48 common varieties. The volume is illustrated by 27 Text-figures and 12 Plates, some of the latter being below expectation, and there is a good index. The text contains 19 Tables, most of which are useful summaries of data; Table IX, which runs to four pages, is an excellent key to the diseases, pests and some conditions of the potato.

The authors state that for various reasons their treatment of the subject is largely restricted to 'such information as is not available at all or, if so, occurs only in the form of articles in scientific journals published in this and other countries'. Their aim is to discuss and summarize this information 'in relation to the many practical problems with which the grower is confronted'. The treatment of material in the book is very unequal, many parts being elementary, whilst others, e.g. Chapter XIX on viruses in relation to potato degeneration and Chapter XX on the spread of virus diseases under field conditions, are highly technical. Also, certain topics that one might expect to find in a work of this title and character are absent. For example, the more botanical chapters contain little reference to the anatomy, development, genetics, physiology and biochemistry of the plant; the husbandry chapters might have given more attention to the modern machinery which is finding increasing use in this crop; and the disease chapters might have included some discussion of potato pests. For instance, Colorado beetle appears once only, in connexion with the resistance of certain wild species of *Solanum*; slugs, capsids and wireworms appear only in Table IX and the two latter are omitted from the index; eelworms are also listed in Table IX, but otherwise receive only three casual mentions in the text; flea beetle and aphids are discussed only in relation to virus transmission. These are all matters of which some notice might be taken when a third edition is called for.

On the whole, however, the authors have done their work extremely well and within its compass—potato varieties, cultivation and diseases—the volume is a rich compendium which everyone interested in this primary crop will find frequent need to consult.

WILLIAM B. BRIERLEY

An Introduction to the Taxonomy and Nomenclature of Fungi. By G. R. BISBY. Pp. vii + 117. Kew, Surrey: The Imperial Mycological Institute, 1945. 5*s*. 0*d*.

This little book is a real gem and will do more to help forward the study of the fungi than a dozen meetings of Learned Societies. I do not know of any other book covering just this ground, and it is a book to be read, marked, learned and inwardly digested not only by every budding student of mycology and plant pathology but by those of us who, having reached years of maturity in teaching, research or advisory work, are supposed to be well acquainted with these matters. If we took to heart Dr Bisby's words of wisdom and clear recommendations, mycology and plant pathology would become much tidier and more satisfactory sciences than they are at present.

Following an introductory chapter on general principles, the book divides into two parts, the first dealing with taxonomy and the second with nomenclature. Part 1 contains ten chapters dealing respectively with the amateur, choice of a group to study, equipment, collecting, examining and recording, measuring, culturing, naming and describing, preserving material, and publishing and illustrating. Part 2 contains five chapters dealing respectively with categories of fungi, synonymy, types and the type method, diagnoses, and the International Rules of Botanical Nomenclature. The book closes with a useful bibliography and an index.

Chapter VII, which deals with the measuring of spores, etc., seems to me to be of particular value. Mycology is primarily a descriptive science and spore measurements are one of the few types of quantitative data which are feasible. To be of value they must be exact and yet it often seems to me that more slovenly and inaccurate work has been published on spore measurements, even by workers of high repute, than in any other branch of mycological study. The only criticism I have to make of this chapter is that it errs, I think, on the side of understatement. Chapter XVI on 'The Rules' occupies nearly one-half of the book. The Articles and Recommendations are given verbatim and incorporate the alterations passed at Amsterdam in 1935, but the names of phanerogamic plants are replaced by examples from the fungi and, throughout, there are illuminating comments by the author. The chapter concludes with Maire's list of *nomina conservanda*. I can imagine no mycologist or plant pathologist who would not benefit from reading this chapter. But the other chapters are all equally good and the whole book is written in simple clear language and with a wealth of cogent and practical illustration drawn from the author's rich experience. I know very few books indeed in which so many valuable suggestions are packed into so small a compass or which bear so clearly the hallmark of first-hand knowledge and experience.

WILLIAM B. BRIERLEY

Plants and Plant Science in Latin America. Edited by FRANS VERDOORN. Pp. xl + 384, with 38 plates and 49 text-figures. Waltham, Mass., U.S.A.: Chronica Botanica Co. (London: Wm. Dawson and Sons, Ltd.) 1945. $6.0.

In our boyhood reading most of us conquered Mexico with Cortes and Peru with Pizarro, we voyaged with Darwin in the *Beagle*, journeyed with Agassiz, von Humboldt and Waterton through Brazil, explored the Amazons with Bates, Spruce and Wallace, collected orchids with Millican in the Northern Andes, travelled with Belt in Nicaragua, with Rodway and Stedman in Guiana, with Hudson in La Plata and Patagonia and dwelt with him in a 'purple land'; and yet, to-day, there are probably few great regions of the world of which we are more ignorant. Within our lifetime this vast romantic 'land of promise' has become a land of scientific achievement but, for perhaps most of us, it remains a continent darker than Africa. And so this fine volume, with its tale of great universities, museums, research stations, botanic gardens, scientific societies, books, journals and all the developments of a major agricultural and scientific efflorescence, will be a landmark in our appreciation of Latin America.

The book consists of some ninety articles ranging from one to seventeen pages in length and dealing with various aspects of botany, agriculture, horticulture and related topics in the countries from Mexico to Argentina, including the West Indian Islands, the Falklands, Galapagos and Juan Fernandez. Many of the eighty-five collaborating authors are United States or European experts with intimate experience of South American problems, whilst others derive from the Latin American countries themselves. The book does not pretend to be in any way a complete survey and the Editor admits that 'its various chapters are often somewhat unequal in concept', but, nevertheless, it gives an admirable bird's-eye view of the plant problems of this vast terrain. It is a sort of botanical *hors-d'œuvre*, interesting and appetizing rather than satisfying—but then, a satisfying meal would demand not one volume but a library, and to this end a wealth of references are cited in the text.

Following an interesting and thought-provoking essay by the Editor on 'The plant scientist in the world's turmoils' and some fifteen pages of references to the general and technical literature of South American botany, the contents are arranged in two parts, the first consisting primarily of new writing and the second of material already published in *Chronica Botanica*, but now appearing mostly in revised form. In each part the articles are grouped into descriptions of the vegetation and plant resources of the several regions from north to south and articles of more general nature, and they cover a very wide range—history, geology, soils, conservation, climatology and meteorology, agriculture, horticulture, forestry, plant distribution and ecology, palaeobotany, ethnobotany, genetics and plant breeding, mycology and plant pathology, economic plants and plant products, educational and research facilities, etc.

Most of the articles are of applied interest and among the longer ones may be noted: 'Some problems of tropical American agriculture', by Popenoe; 'Principal economic plants of tropical America', by Fosberg; 'Les conditions écologiques, la végétation et les ressources agricoles de l'archipel des petites Antilles', by Stehlé; 'Hevea rubber culture in Latin America, problems and procedures', by Rands; 'Fat and oil resources of Latin America', by Markley; 'The location of botanical collections from Central and South America', by Lanjouw (in which the explanation of the symbols used to designate the herbaria is not included; the Editor states that it will 'soon be published in *Chronica Botanica*'; 'Plant breed-

ing, genetics and cytology in Latin America', by Krug; 'Conservation in the Americas', by Coolidge, and a most useful article by F. and J. G. Verdoorn on the 'Plant science institutions, stations, museums, gardens, societies and commissions in Central and South America'. Very interesting but less applied articles are Pennell's 'Historical sketch' of botanical exploration in Latin America, and Hill's 'Ethnobotany in Latin America'. Most of the shorter articles also contain valuable information which cannot easily be obtained elsewhere.

The volume is of most pleasing format and a special word of praise may be given to the illustrations which are of rather unusual quality, many being reproduced from rare or classical works.

The only criticisms I would make of the book concern the arrangement of its contents and the absence of a subject index. Instead of the division into two parts according to whether the work had or had not previously been published—which is immaterial to the reader—it would have been more convenient if all the general articles had been collected together and followed by the regional descriptions in alphabetical order of their countries. Regarding the indices the Editor states that 'it was not feasible to prepare a subject index for our polyglot volume' but, for the many readers more interested in a specific plant or problem than in the vegetation of a particular region, the lack of a subject index renders the volume almost unusable without an exasperating waste of time. In this book of over 400 pages, some forty pages are in Spanish and Portuguese and fifteen in French, so that the book is presumably intended for English-speaking readers, few of whom are familiar with the Iberian languages. Is it too much to hope that in the second edition, that will surely be called for, the odd fifty-five pages may be translated into English? This would make the book more useful, remove the polyglot bar to the preparation of a subject index and, at the same time, render unnecessary the very lengthy and confusing Table of Contents. WILLIAM B. BRIERLEY

Hayfever Plants: their Appearance, Distribution, Time of Flowering, and their Role in Hayfever, with special Reference to North America. By R. P. WODEHOUSE. Pp. xix + 245. Waltham, Mass., U.S.A.: the Chronica Botanica Co. (London: Wm. Dawson and Sons, Ltd.) 1945. $4.75.

Hayfever is one of the more distressing minor complaints and in most cases it is directly traceable to contact of airborne pollen with the mucous membranes of the eyes and upper respiratory tract of susceptible persons. It is, therefore, very desirable to know as much as possible about the pollens concerned, their appearance so that they can be identified, their abundance, buoyancy, allergenic toxicity, the seasonal incidence of pollen shedding and the regional distribution of the relatively few plant species which are involved.

Chapter 1 is devoted to the more general botanical issues, including the methods of air-sampling for pollen, and concludes by the citation of relevant literature. In chapters 2 and 3 the hayfever plants of North America, arranged according to Engler and Prantl, and their pollen grains are described and illustrated by original drawings of the plants or their flowering parts and their pollen grains. Chapter 4 is the most practically important section of the book and in it are collated some 135 regional surveys made by various people. The author divides the United States into ten areas which are considered seriatim and in each of which the occurrence and distribution of the specific hayfever plants and their times of flowering and pollen shedding are correlated with the geographic and seasonal incidence of the complaint. The discussion of each area terminates with references to the special literature of that area and notes on local health resorts where sufferers may obtain relief. On p. 186 there is a brief reference with literature citations to the interesting recent work on mould spores which 'play an important part in asthma and hayfever of the South, though perhaps not more so than elsewhere'.

The book concludes with a glossary of botanical terms, a useful bibliography and author and general indexes. It is pleasantly produced and the frontispiece is a reproduction of one of the beautiful plates from Fritzche's *Ueber den Pollen* published in 1837. WILLIAM B. BRIERLEY

Chromosome Atlas of Cultivated Plants. By C. D. DARLINGTON and E. K. JANAKI AMMAL. Pp. 397. London: George Allen and Unwin Ltd. 1945. 12*s.* 6*d.*

This book opens with a very interesting Introduction of twenty-eight pages by Darlington in which he combines the views of De Candolle, Darwin and Mendel on plant origins and genetic behaviour with Vavilov's work on centres of dispersal and the chromosome studies of modern geneticists, and uses the key thus forged to unlock the problems of the evolution, distribution, systematics and improvement of cultivated plants. It is interesting to compare Darlington's views with those recently expressed by Hudson in his valuable paper on 'Plant breeding and genetics to-day' (*The Advancement of Science*, 1945, **3**, 352). Darlington's Introduction is really a sketch for a larger work which one hopes he will soon find time to write.

The book proper consists of 317 tightly packed pages of lists in parallel columns of scientific and popular names, chromosome numbers and their authors, and the uses and distribution of wild and cultivated plants. The families and tribes, inserted as centre headings, mostly but not entirely follow Hutchinson's published and unpublished arrangements. Under each family or tribe the genera with their species are listed in column one. The tribes and genera, and within each genus one or more groups of species, are arranged in order of ascending basic chromosome numbers whilst within each group of species the names run alphabetically. This arrangement of botanical names makes it quite impossible to consult the data for any one plant without turning every time to the index and the situation is aggravated by the fact that, in all the systematic categories, exceptions are admitted by the authors to their standard arrangement. A simple alphabetical tabulation by genera and by species would have made the book much easier to use merely as a key to chromosome numbers, but it would have completely obscured the main purpose of the authors which is to exhibit the fundamental relationship between chromosome numbers and the botanical classification of plants. The classification which eventuates from this relationship

and which is crystallized in the authors' arrangement merits and will certainly receive the closest scrutiny by systematists and geneticists. It is a most illuminating approach to the problems of plant classification and evolution and whilst it clears up many problems it opens up innumerably more and will be most stimulating to further research in this primary field of botanical study.

The second column lists the popular names of most of the important economic and decorative plants. Although the authors state that they 'have tried (with partial success) to avoid the many bogus popular names' some of those adopted are certainly not the best known or most widely used. A number of them I have entirely failed to trace in any other botanical work so that one feels they almost merit the term 'bogus'. To instance a few from our common weeds: how many botanists would recognize *Ranunculus sceleratus* as 'blister buttercup', *Nymphaea alba* as 'flatterdock', *Chenopodium album* as 'pigweed', *Erodium cicutarium* as 'pin grass', *Urtica urens* as 'dog nettle', *Impatiens biflora* as 'jewel weed', *Stachys sylvatica* as 'clownwort', or *Elodea canadensis* as 'ditch moss'?

Column 3, which lists the 2 X-chromosome numbers of the plants, is, of course, the *raison d'être* of the book. Some 8400 species are recorded in detail, about 2000 more are summarized without individual names owing to their uniformity of number or lack of cultural interest, whilst another 1000 or so are included although their chromosome numbers are unknown. Many well-known species do not appear in the list and it is a little difficult to understand their exclusion in view of the presence of others of considerably less importance. The fourth column states the authors of the numbers with the date of publication.

The fifth column is a list of symbols, explained earlier in the text, by which the plants are classified into one or more of thirty categories according to the economic usefulness of the plant or its products. The last column indicates the distribution of the plants based primarily upon the *Index Kewensis* whose main geographical divisions are shown on the back end-papers. Judging from the text, the front end-papers, which show Vavilov's centres of dispersal, and the back end-papers have been reversed. It is gradually becoming clear that natural polyploidy and plant evolution are to some extent related to geographical distribution since the proportion of polyploid species is especially high in the flora of lands with rigorous climates, and these columns of apposed chromosome numbers and distribution will be valuable in the examination of this problem. The volume closes with a Bibliography of over 1000 references, including citations of the year 1944, and an Index to Families and Genera.

From time to time most of us have had occasion to consult the folios of *Tabulae Biologicae* or the lists compiled by Ishikawa, Gaiser and others to find out the chromosome number of some particular plant; to consult the volumes by Burkill, Holland, Rehder, Sampson, etc., to discover its economic uses; or to wade through the *Index Kewensis* and various floras to ascertain its geographical distribution; and we have all lamented the time thus absorbed. To have all these data collected and arranged in systematized and convenient form within one volume makes this book almost indispensable for workers in all branches of 'pure' and applied botany. The only criticisms one has to make of the book are that there is no space on the pages for one's own annotations and additions, and in a book of this type everyone will wish to make annotations and additions, and that the volume is produced and bound in a rather flimsy style which will scarcely support the hard usage it is likely to receive. On the other hand, it is surprising and very gratifying that a work of such magnitude and scientific value could have been prepared and produced at all in this country during the stresses of wartime. Its compilation must have been a Herculean task and a very 'labour of love', and its authors have earned the gratitude of botanists the world over.

WILLIAM B. BRIERLEY